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14. ABSTRACT

We have successfully submitted clinical trial documents to regulatory agencies following a long interaction with the DOD following their approval of a three year clinical trial award.. We have received an exemption from the RAC and are awaiting final approval by the DOD's HSRRB, the University of Iowa's IRB, and the FDA.

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Vaccine Immunotherapy for Prostate Cancer
David M. Lubaroff, PhD
Principal Investigator
Grant Number PC050647
Final Addendum Report

INTRODUCTION: The goal of this Clinical Trial Developmental Award (CTDA) was to develop and complete the required administrative tasks necessary to begin a Phase II clinical trial of an adenovirus/PSA (Ad/PSA) vaccine in men with recurrent prostate cancer and a Phase I clinical trial of the combination of the Ad/PSA vaccine along with immunostimulatory CpG ODN. The tasks included the design and construction of the clinical protocols, informed consent forms, submission to the institutional committees that include the Protocol Review and Monitoring Committee, Human Subjects Committee (IRB), Biosafety Committee, Pharmacy and Therapeutics Committee; the NIH Recombinant DNA Advisory Committee (RAC), and the food and Drug Administration (FDA). In addition, although not funded by the CTDA, we were required by the FDA to perform Pharmacology/Toxicology and Histopathology Studies and to obtain a complete DNA sequence of the Ad/PSA vaccine.

BODY: We describe below the accomplishments during the one year extension of the award, using the original Statement of Work tasks. The accomplishments include those previously reported at the end of the original one year award.

Sequence the Ad5-PSA vaccine – The clinical grade Ad/PSA vaccine was submitted to Lark Technologies. The sequence report indicated that there were no additions to the normal sequences of the PSA insert nor to the adenovirus backbone.

Develop protocols, investigator brochures, and IRB forms for the Phase II trial of the Ad/PSA vaccine alone – This task consumed the great majority of time during the first year of the award. Multiple meetings were held among the Principal Investigator (PI) and members of the Clinical Trial Team to make important decisions about the trial protocols. The discussion point included the patient populations to be targeted in the trials, the eligibility and exclusion criteria, the primary and secondary endpoints, and times for follow-up visits. The plans for the trial were presented to large groups of basic scientists and clinicians, the results of which enhanced the final protocols. In addition, a number of meetings were held during the year with two biostatisticians in the Biostatistics Core of the Holden Comprehensive Cancer Center. In the end, two protocols, targeting three separate patient populations, were completed for the Phase II trial. The Investigator's Brochure was also constructed. We submitted applications to Holden Comprehensive Cancer Center's the Protocol Review and Monitoring Committee, as well as the University of Iowa's Pharmacy and Therapeutics and Biosafety Committees. All submissions were approved. An application to the NIH RAC was submitted and we received a notice that we are exempted from full committee and public review of the protocols.

An application for a Clinical Trial Award was submitted to the DOD's Prostate Cancer Research Program and was funded following receipt of an e-mail notification on October 11, 2006. On December 12, 2006 the PI, Co-PI, and Data Manager for the award and trial were required to attend a pre-award meeting and protocol workshop at Fort Detrick, Maryland. At this meeting we received documents from a reviewer in the Office of Research Protection (ORP) to which we responded by January 8, 2007. We have been working with the ORP and the Human Subjects Research Review Board (HSRRB) to develop the final documents to the University of Iowa's IRB and to the FDA. The process has been protracted with multiple requests from the initial ORP reviewer who was subsequently terminated from her employment to be replaced by a

second reviewer with whom we have worked closely. We also participated in a telephone conference call with the HSRRB followed by additional requests for revisions to the protocols, informed consent documents, and the letter to be used for patient recruitment. We received provisional approval by the HSRRB, pending approvals from the IRB and FDA. On August 16, 2007 we received confirmation that there would not be any further requirements for change sin our trial documents. On this date we submitted the documents to the IRB and FDA.

Initial submissions were made to the IRB and FDA in November 2006. The FDA responded with requests for minor changes, mostly based on the elaboration of statements made in the protocols and in the data submitted from the pharmacology/toxicology studies (see the following section). Neither the IRB or FDA would consider further review until all issues were settled with the DOD and final protocols, informed consent documents, as well as other supporting materials were produced. On August 16, 2007 the final documents were submitted to the respective agencies. Approvals are pending.

Develop protocols, investigator brochures, and IRB forms for the Phase I trial of the Ad/PSA plus CpG ODN – This process has been delayed due to the ongoing negotiations between our clinical trial team, Coley Pharmaceuticals Group, and Pfizer, Inc. Coley was the company that initiated clinical trials of the immunostimulatory CpG ODN, but has since out-sourced all of their cancer vaccine work to Pfizer. Thus, until an agreement can be reached, we have delayed our Phase I trial of the Ad/PSA plus CpG ODN.

Analyze data from FDA-required required studies (not funded by the Clinical Trial Development Grant) – We are required to perform pharmacology/toxicology and histopathology studies in mice injected with the three injection prime-boost strategy we propose for the Phase II trial. Mice were injected with 10^8 pfu of the Ad/PSA vaccine in a collagen matrix, a dose determined by the results of our completed Phase I trial. At each of the 8 follow-up time periods after vaccine injections for the study – days 0, 30, 33, 44, 60, 63, 74, and 90, we examined the injection site for evidence of local toxicity, bled the mice, and removed selected organs for study as described below. We also examined the mice daily for overt signs of clinical toxicity such as ruffled fur, posture, and activity. Each mouse was weighed twice per week. Blood was collected and used to measure AST, ALT, LDH, alkaline phosphatase, bilirubin, glucose, total protein, BUN, and creatinine. Tissues that were analyzed for histopathology: injection site, draining lymph node, contralateral lymph node, liver, lung, spleen, heart, brain, gonads, prostate, urinary bladder, kidney. The data obtained fro these studies demonstrated that no abnormalities were found in any of the 11 tissues examined and normal values were evident throughout the study period in the analyses for blood cells, renal function, and liver function.

Arrange pre-IND conference for the Phase I trial – Postponed as the result of delayed negotiations with Pfizer. See explanation above.

Prepare and submit IND application for Phase I trial - Postponed as the result of delayed negotiations with Pfizer. See explanation above.

Receive approval from the FDA for Phase I trial - Postponed as the result of delayed negotiations with Pfizer. See explanation above.

Prepare and submit amendment to current IND for Phase II trial – This task has been completed and described in a previous section of this report.

Receive approval from the FDA for Phase II trial – Pending.

KEY RESEARCH ACCOMPLISHMENTS:

- Completed two clinical protocols for the Phase II trial of the Ad/PSA vaccine
- Submitted protocols to University of Iowa institutional committees – Protocol Review & Monitoring, IRB, Biosafety, and Pharmacy & Therapeutics and received approvals
- Prepared and submitted application to NIH RAC.
- Received exemption from full RAC review of the protocols
- Completed modifications to clinical trial documents as requested by the DOD's ORP and HSRRB.
- Submitted documents for approval to the University of Iowa's IRB and to the FDA. Approvals pending.
- Sequenced the DNA of the vaccine.
- Performed pre-clinical pharmacology/toxicology and histopathology studies.
- Began negotiations with Pfizer, Inc. for joint Phase I clinical trial of the Ad/PSA vaccine plus CpG ODN.

REPORTABLE OUTCOMES INCLUDED IN APPENDICES: Attached are two clinical protocols, informed consent documents, and letter for patient recruitment.

CONCLUSION: We have successfully submitted clinical trial documents to regulatory agencies following a long interaction with the DOD following their approval of a three year clinical trial award.. We have received an exemption from the RAC and are awaiting final approval by the DOD's HSRRB, the University of Iowa's IRB, and the FDA.

Individuals Who Received Salaries:

1. David M. Lubaroff, PhD, Principal Investigator
2. Marjorie Kuperman, Office Assistant (left employment in January 2007 for health reasons).
3. Diane Morman, Office Assistant
4. Jennifer Paisley, Research Assistant

PHASE II STUDY OF ADENOVIRUS/PSA VACCINE IN MEN WITH RECURRENT PROSTATE CANCER AFTER LOCAL THERAPY

Food and Drug Administration (FDA) Investigational New Drug (IND) #9706
Department of Defense, Prostate Cancer Research Program #A-14059.1
Version 32 – August 15, 2007

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The Principal Investigators are funding this clinical trial through an award from the Department of Defense's Prostate Cancer Research Program.

[1] Please send all communications to this investigator.

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1. INTRODUCTION

1.1 Background- Immunotherapy in Prostate Cancer

Prostate cancer is the second leading cause of cancer death among males in the United States. There will be an estimated 234,460 new diagnoses of prostate cancer made in the United States in 2006 (1) (the estimates for 2006 were not available at the time of the protocol completion). Treatments for organ-confined prostate cancer include radical prostatectomy and radiation therapy. When the cancer presents de novo, or recurs outside the prostate, first-line systemic treatments typically include hormonal blockade (with LHRH agonists or bilateral orchiectomy), which suppress testosterone levels, limit the growth of androgen-dependent cancer cells, and result in clinical tumor control. After a median time of 2 years, patients progress into a clinical hormone-refractory state, when the prostate specific antigen (PSA) levels rise despite castration, there is proliferation of androgen-independent cancer cells, and there is continued clinical tumor growth that becomes fatal. Therapeutic measures in this situation include further hormonal manipulations or the use of systemic chemotherapy, which has recently shown a small survival benefit in phase III trials. Approximately 30,000 Americans die from prostate cancer each year.

Immunotherapeutic approaches against prostate cancer have been investigated for several years. Most of these studies have concentrated on active non-specific therapy and adoptive or passive therapy, with only recent focus on the induction of antigen-specific immune responses. Viral vectors have been used successfully in both gene transfer and vaccine therapy studies (2). Replication-competent and replication-deficient adenoviruses expressing foreign proteins have been used to elicit immune responses to a variety of tumor antigens (3-7).

We have demonstrated that immunizations with adenovirus, carrying the human PSA gene, can induce vigorous anti-PSA T-cell responses and cause the destruction of PSA-secreting tumors in a pre-clinical mouse model of prostate cancer (8,9). Such active immunization against prostate-cancer associated antigens might be more effective than active non-specific or adoptive/passive immunotherapy. Therefore, we have pursued a vaccination strategy based on an adenovirus that carries the gene for prostate specific antigen (PSA). Results from our Phase I trial of adenovirus/PSA (Ad/PSA) vaccine (section 1.4, below) demonstrated that a single immunization of men with metastatic prostate cancer was able to induce anti-PSA T cell responses. The trial design was a dose escalation study with the vaccine administered subcutaneously (sc) either in an aqueous solution or in a collagen matrix (Gelfoam®). We now propose a Phase II clinical trial using the Ad/PSA vaccine, administered in multiple injections to prostate cancer patients with minimal disease burden.

1.2 Adenovirus vectors

Recombinant adenoviral vectors transduce a wide range of dividing and nondividing cells types, making this gene delivery system valuable as a tool for studying diseases, for vaccine therapy, and for potential clinical use (10). Recombinant adenovirus can be prepared and purified in high titers. In addition, wild-type adenovirus infections are extremely common in the general population, giving adenovirus a well-documented safety record (11). Moreover, adenoviruses are structurally stable and no adverse effects have been reported following the vaccination of US military recruits with wild types, demonstrating their safety for human use (11). Adenoviral vectors for gene therapy and vaccine therapy are adenoviruses which have been genetically modified to allow insertion of foreign genes and to render the virus replication-defective. Current vectors have a deletion in the E1 region or in both the E1 and E2 regions.

Adenoviral gene transfer has been used in a variety of experimental conditions that include transfers to the liver (12), lung (13), central nervous system (14,15), and to cancer cells (16).

There is evidence that the introduction of foreign transgenes by adenovirus induces immune responses to the transgene product, which become ultimately responsible for the elimination of the virus (17,18). While this is disadvantageous for insertion of functional genes into host cells, it is advantageous in the use of viruses carrying foreign genes as immunogens. In the vaccine therapy of cancer, active immunization against a murine colon cancer, breast cancer, and melanoma antigens have been induced by adenoviral vaccines (19-25).

The Ad/PSA vaccine used our laboratory and in our Phase I clinical trial was produced by inserting the gene for the full length pre-pro form of human PSA into a replication deficient adenovirus serotype 5. Replication deficiency was induced by deletion of the E1a and E1b genes of the virus. Details of the vaccine can be found in section 9 of this protocol. Approval for the use of the vaccine in the Phase I trial was obtained from the FDA under IND #9706.

In pre-clinical studies, our group has demonstrated that the Ad/PSA vaccine was able to induce stronger anti-PSA immune responses than other viral PSA vaccines. These include vaccinia viruses, both replication competent and replication deficient, and to a canarypox vaccine (Table 1), The frequency of PSA-specific CD8+ cells T cells generated by the Ad/PSA vaccine was greater than were generated by any of the other vaccines tested.. In addition to the superior immunizing property of the Ad/PSA, the incorporation of Gelfoam, a collagen matrix (section 1.3), has been shown in pre-clinical studies to enhance the ability of the vaccine to induce strong anti-PSA immune responses (8). Lastly, immunization of mice with Ad/PSA in matrix can induce anti-PSA responses even in the presence of high titer anti-adenovirus antibodies (8). This latter finding is important in light of the fact that most humans have pre-existing levels of anti-adenovirus antibodies as a result of prior natural exposure to the virus.

Table 1
Effector Cell Frequency Analysis (ELISPOT)

Vaccine	Virus	Frequency of PSA-Specific CD8+ T Cells
Ad/PSA*	Replication deficient adenovirus	1/455
Prostvac	Replication competent vaccinia	1/2028
NYVAC/PSA	Replication deficient vaccinia	1/3597
ALVAC/PSA	Canarypox	1/35,714

1.3 Gelfoam® Matrix

Gelfoam (Pharmacia & Upjohn Company, Kalamazoo, MI) is a medical device intended for application to bleeding surfaces as a hemostatic agent. It is a water-insoluble, off-white, non-elastic, porous, pliable product prepared from purified pork skin. The Gelfoam gelatin preparation is available either as a cross-linked sponge or as non-cross linked beads. It is able to absorb and hold within its interstices approximately 45 times its weight of blood and other fluids (26). The absorptive capacity of Gelfoam is a function of its physical size, increasing with increasing gelatin volume (27).

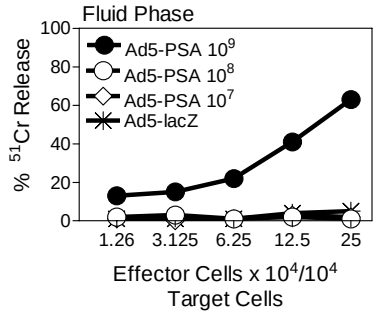
The mechanism of action of surface-mediated hemostatic devices is supportive and mechanical (27). Surface-acting devices, when applied directly to bleeding surfaces, arrest bleeding by the formation of an artificial clot and by producing a mechanical matrix that facilitates clotting (28). Jenkins et al have theorized that the clotting effect of Gelfoam may be due to release of thromboplastin from platelets, occurring when platelets entering the Gelfoam become damaged by contact with its myriad of interstices (29). Thromboplastin interacts with prothrombin and calcium to produce thrombin, and this sequence of events initiates the clotting reaction. The authors suggest that the physiologic formation of thrombin in Gelfoam is sufficient to produce formation of a clot, by its action on the fibrinogen in blood (29). The spongy physical properties of Gelfoam hasten clot formation and provide structural support for the forming clot (28,30).

Gelfoam has been used experimentally for the delivery of soluble proteins and drugs, including insulin, antibiotics, and growth factors (31-33). Gelfoam was used for sustained release of insulin in an ocular implant device (31). Delivery of insulin in solution had no effect on blood glucose levels. In contrast, the use of Gelfoam as a sustained release delivery agent provided measurable insulin activity for up to 10 hours after implantation. Glucose levels in the blood stabilized at 60% of the original value, whereas administration of insulin in eye drops had no effect.

MacDonald and Mathews (34) studied Gelfoam implants in canine kidneys and reported that it assisted in healing, with no marked inflammatory or foreign-body reactions. Jenkins and Janda (35) studied the use of Gelfoam in canine liver resections and noted that Gelfoam appeared to offer a protective cover and provide structural support for the reparative process. Correll et al (36) studied the histology of Gelfoam when implanted in rat muscle and reported no significant tissue reaction.

Gelfoam has been used as a hemostatic agent in dog prostate (37). In these studies no gross histological evidence of tissue damage or calcification was induced. In addition, these investigators demonstrated that placement of Gelfoam into the lumen of the bladder resulted in liquefaction of the Gelfoam without any evidence of calculogenesis. Finally, Bischoff and Goerttler (38) used Gelfoam in human prostate therapeutic embolization with success.

Our laboratory, in collaboration with Dr. Timothy Ratliff, has demonstrated that administration of the Ad/PSA vaccine in Gelfoam induces a stronger anti-PSA immune response (Figure 1). In our pre-clinical studies, immunization with the vaccine in an aqueous suspension induces strong immunity with 10^9 pfu with weaker immunity induced with 10^8 and 10^7 pfu. Use of Gelfoam permits the induction of strong responses at the lower dose of 10^8 pfu. In addition, strong anti-PSA T cell responses could be induced by immunization with the Ad/PSA vaccine in Gelfoam even in mice pre-immunized to adenovirus (Figure 2). In the Phase I clinical trial (section 1.4), the addition of Gelfoam to the vaccine immunization did not result in excess serious adverse events.



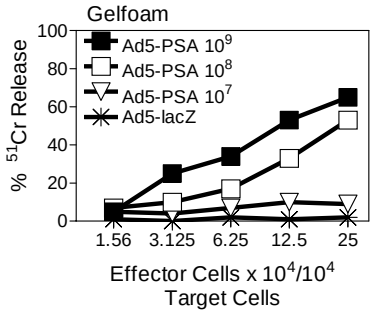


Figure 1 – Anti-PSA cytotoxic activity of spleen cells obtained from mice immunized with Ad/PSA in aqueous (fluid phase) versus Gelfoam.

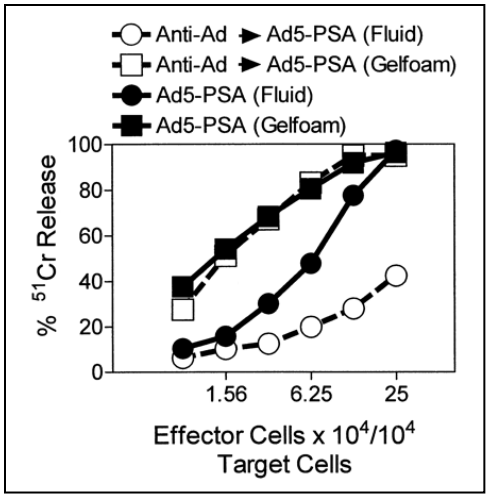


Figure 2. Effect of the presence of anti-adenovirus Ab on Ad5-PSA immunization with and without Gelfoam. Mice were injected i.p. with 10^9 PFU of Ad5-lacZ or PBS 2 wk before immunization with 10^9 PFU Ad5-PSA delivered s.c. in the fluid phase (PBS) or Gelfoam. CTL were tested against RM-11psa and RM-11neo to demonstrate PSA specificity. Cytolytic activity of each group against RM-11neo targets was <10% for all E:T ratios.

1.4 Phase I study

A Phase I clinical trial of the Ad/PSA vaccine has been completed in men with measurable metastatic prostate cancer, with the primary objectives of determining the toxicity profile and maximal tolerated dose (MTD). The ability of the vaccine to induce anti-PSA immune responses and any clinical responses was also evaluated. Funding for the Phase I trial was provided by multiple sources that include the Holden Comprehensive Cancer Center, the Department of Urology, and the Carver College of Medicine at the University of Iowa.

Eligible patients consisted of men with prostate cancer that had measurable metastatic disease, 90% of whom were stage D3. Prior therapies had included androgen withdrawal, ketaconazole, prednisone, Casodex, Taxotere, and external beam radiation, but the initiation of vaccine therapy was equal to, or greater than, 30 days after the most recent therapy. Patients were treated in successive dose levels and aqueous vs. matrix cohorts, according to the protocol plan. We were able to administer the maximum permitted dose of 10^8 pfu without any serious adverse events by vaccination of the first of 18 patients. These initial 18 patients were followed throughout the one-year period after injection. An additional 14 patients were treated at the MTD dose level, as planned in the protocol and confirmed by a letter to the FDA. The purpose of the additional patients was to have sufficient numbers of patients in the groups to statistically evaluate the anti-PSA immune responses induced by the Ad/PSA vaccine. In summary, 32 patients were treated in the study followed through the one-year period. Two additional patients were enrolled in the study (total number of enrolled patients, 34) but never received the vaccine and were therefore not evaluable. One patient chose to have radiation therapy instead of participating in the trial and the second was diagnosed with a second malignancy (melanoma) shortly after his enrollment in the phase I study.

1.4.1 **Phase I study results**

The median age of the patients was 70.2 years (range, 52 to 89). The vaccine was administered as an aqueous suspension or in a collagen (Gelfoam) matrix to 32 patients. Sixteen (16/32) or 50% of the patients exhibited grade 1 vaccine-related adverse events (AE), 1/32 (3.1%) that exhibited a grade 2 AE, and one patient exhibited a grade 3 AE which was a decrease in neutrophil count. There were no vaccine-related grades 4 or 5 AEs.

Table 2
Ad/PSA Phase I Trial
Adverse Events – by Vaccine Relationship

Patient	System	Day of onset*	Event	Vaccine-related
AP005	Neural	21	Agitation	No
AP006	Musculoskeletal	13	Left hip and thigh pain	No
AP007	Musculoskeletal	22	Left back pain	No
AP007	Neural	14	Situational depression	No
AP009	GI	21	Constipation	No
AP009	Musculoskeletal	14	Joint aches, secondary to fall	No
AP009	Neural	11	Fall, causing head lacerations & loss of consciousness	No
AP010	GU	21	Proteinuria	No
AP010	Skin	21	Edema	No
AP014	Constitutional	22	Fatigue	No
AP014	Musculoskeletal	14	Leg pain (bone – femur)	No
AP015	Respiratory	1	Cough	No
AP016	GU	172	Left nephrolithiasis	No
AP016	GU	183	New primary tumor – papillary bladder	No
AP016	Immune	8	Flu-like symptoms	No
AP016	Musculoskeletal	4	Bone pain – left leg	No
AP016	Musculoskeletal	21	Decreased left leg strength	No
AP018	Musculoskeletal	8	Bilateral rib pain	No
AP018	Neural	8	Insomnia	No
AP020	Neural	168	cord compression	No
AP022	GI	2	constipation	No
AP022	Hematologic	14	decreased lymphocyte count	No
AP022	Hematologic	63	decreased WBC & neutrophils	No
AP022	Musculoskeletal	5	increased bone pain	No
AP022	Neural	16	depression	No
AP023	Musculoskeletal	20	pain in left buttocks	No
AP025	Musculoskeletal	16	bone pain	No
AP026	Cardiovascular	14	Lower extremity edema	No
AP027	GI	1	GI cramping	No
AP027	GI	1	constipation	No
AP034	Respiratory	prior	Edema, wheezing (change in inhaler in same time period)	No
AP035	Constitutional	64	Weight loss	No
AP035	GI	50	Constipation	No
AP035	GU	22	Proteinuria	No
AP035	Hematologic	15	Lymphopenia	No
AP035	Musculoskeletal	22	Increased alkaline phosphatase	No
AP037	GI	73	Nausea	No
AP018	Skin	1	Injection site tenderness	Possible
AP019	Cardiovascular	1	Hypotension	Possible
AP019	Constitutional	1	fever	Possible
AP019	Constitutional	14	fatigue	Possible
AP019	GU	1	Proteinuria	Possible
AP020	GI	21	increased alkaline phosphatase	Possible
AP020	GU	21	ketonuria	Possible

AP020	Metabolic	14	hyponatremia	Possible
AP020	Metabolic	14	hyperglycemia	Possible
AP020	Respiratory	9	viral symptoms	Possible
AP005	Musculoskeletal	1	Left inguinal pain	Possible
AP008	GU	21	Proteinuria	Possible
AP008	Respiratory	3	Cold symptoms	Possible
AP010	Hematologic	1	Decrease in absolute neutrophils count	Possible
AP027	Liver	21	increased AST	Possible
AP036	Hematologic	1	Anemia	Possible
AP036	Hematologic	63	Anemia	Possible
AP037	Hematologic	1	Lymphopenia	Possible
AP002	Musculoskeletal	3	Groin pain	Unlikely
AP002	Neural	3	headache	Unlikely
AP002	Skin	1	Itching	Unlikely
AP003	Pulmonary	21	Pleural effusion	Unlikely
AP006	Constitutional	14	Chills	Unlikely
AP006	Neural	14	Migraine headache	Unlikely
AP012	Cardiovascular	21	Hypotension	Unlikely
AP012	GI	0	Heartburn	Unlikely
AP012	Neural	21	Dizziness	Unlikely
AP014	Hematologic	82	Anemia	Unlikely
AP015	Cardiovascular	137	Myocardial infarction	Unlikely
AP018	Respiratory	43	DOE?	Unlikely
AP021	Hematologic	1	decreased lymphocytes	Unlikely
AP021	Hematologic	63	thrombocytopenia	Unlikely
AP023	Immune	16	urinary tract infection	Unlikely
AP023	Liver	21	elevated AST	Unlikely
AP024	Hematologic	14	anemia	Unlikely
AP024	Hematologic	1	anemia	Unlikely
AP025	GI	85	abdominal distention, constipation, apparently related to metastatic disease in the periaortic lymph nodes and periprostatic tumor mass	Unlikely
AP025	Hematologic	14	thrombocytopenia	Unlikely
AP025	Neural	122	Headache	Unlikely
AP027	Immune	21	Infection	Unlikely
AP034	Cardiovascular	14	Hypotension/dizziness	Unlikely
AP034	Musculoskeletal	14	Back pain	Unlikely
AP036	Cardiovascular	63	Edema – bilateral ankles	Unlikely
AP002	Skin	0	hematoma at injection site	Yes
AP003	Skin	0	Bruising at injection site	Yes
AP004	Skin	0	Ecchymosis at injection site	Yes
AP005	Skin	0	Ecchymosis and erythema at injection site	Yes
AP008	Skin	0	Ecchymosis at injection site	Yes
AP009	Skin	0	Ecchymosis at injection site	Yes
AP018	Skin	0	Ecchymosis	Yes
AP025	Skin	0	Ecchymosis at injection site	Yes
AP026	Skin	0	Erythema at injection site	Yes
AP034	Skin	0	Pain at injection site	Yes

* day 0 = day of injection; day 1 = day after injection

We measured the anti-PSA immune responses, both antibody and T cell, in all patients enrolled in the study. Antibody responses to PSA were measured by the binding to PSA-secreting cell lines using the method adapted from Cavacini, et al. (39). Results of those analyses demonstrated that 57% of men immunized with the Ad/PSA vaccine developed measurable anti-PSA antibodies. ELISPOT assays were utilized to measure anti-PSA T cell responses. The results, depicted in Table 3, demonstrate that of the 32 patients, 18 (56.3%) developed anti-PSA T cell responses. The addition of Gelfoam did not appear to affect the development of anti-PSA responses, but in this Phase I study the numbers of patients in each group was too small to make statements of statistical significance of the data. These results demonstrate the ability of men with late stage metastatic prostate cancer, injected one time with Ad/PSA, to respond to the vaccine with the production of anti-PSA T cells.

Table 3
ELISPOT Analysis of Anti-PSA T Cell Immune Responses

Patient Number	Dose/ Vehicle	Response	Frequency	
			Pre-Immunization	**Post- Immunization**
AP-002	10^6-aqueous	-	1/985,000	1/258,571
AP-004	10^6-aqueous	-	0	1/311,111
AP-007	10^6-aqueous	+	1/46,901	1/14,804
AP-003	10^6-matrix	+	1/90,000	1/8075
AP-005	10^6-matrix	+	$1/1.2x10^6$	1/152,353
AP-006	10^6-matrix	+	1/93,103	1/15,762
AP-008	10^7-aqueous	-	1/100,000	1/97,419
AP-010	10^7-aqueous	-	0	0
AP-013	10^7-aqueous	+	0	1/60,000
AP-009	10^7-matrix	+	1/52,535	1/30,566
AP-012	10^7-matrix	-	1/13,488	1/254,286
AP-014	10^7-matrix	+	1/562,500	1/120,000
AP-015	10^8-aqueous	+	$1/1.4x10^6$	1/780
AP-016	10^8-aqueous	+	0	1/130,000
AP-017	10^8-aqueous	+	0	1/124,375
AP-025	10^8-aqueous	-	0	0
AP-026	10^8-aqueous	-	0	1/560,000
AP-027	10^8-aqueous	+	0	1/26,364
AP-029	10^8-aqueous	+	0	1/333
AP-032	10^8-aqueous	+	1/250,000	1/3793
AP-018	10^8-matrix	-	0	0
AP-019	10^8-matrix	+	1/14,235	1/336
AP-020	10^8-matrix	+	1/8824	1/1802
AP-021	10^8-matrix	+	1/689	1/431
AP-022	10^8-matrix	+	0	1/180,000
AP-023	10^8-matrix	-	1/320,000	0
AP-030	10^8-matrix	+	1/666.667	1/32,000
AP-034	10^8-matrix	+	1/80,000	1/15,152
AP-035	10^8-matrix	+	1/5295	1/2459
AP-036	10^8-matrix	+	1/62,000	1/9487
AP-037	10^8 matrix	-	$1/2.1x10^6$	1/965,000

The effects of vaccination on serum PSA and on patient survival were also evaluated as a secondary endpoint in the phase I trial. Although there was no sustained decline in individual PSA levels, the PSA doubling times (PSADT, calculated based on 3 pre-enrollment consecutive

PSA measurements) were reduced in 54 percent of the patients compared to pre-vaccine administration, with the best responses occurring in patients immunized with the highest dose of the vaccine (Table 4)**.** In addition, published survival nomograms for patients with hormone refractory prostate cancer were applied to patients in this phase I trial (40,41). Table 5 shows that 57% of all patients at all doses, whether injected with the vaccine as an aqueous suspension or in the collagen matrix, had a survival time longer than that predicted by the nomogram. The range of increased survivals in the different groups was 33% to 100%.

Table 4
PSA Doubling Time (DT) in Phase I Patients

Vaccine Dose & Vehicle	Percent Increased PSA DT
10^6 aqueous	33%
10^6 matrix	33%
10^7 aqueous	67%
10^7 matrix	67%
10^8 aqueous	25%
10^8 matrix	62%
Total	**54%**

Table 5
Ad/PSA Phase I Trial
Predicted and Actual Patient Survival

Vaccine Dose	Vehicle	Percent with Longer Than Predicted Survival
10^6	Aqueous	33%
10^6	Matrix	67%
10^7	Aqueous	100%
10^7	Matrix	33%
10^8	Aqueous	56%
10^8	Matrix	50%
Overall		**57%**

1.5 Other immunotherapy clinical trials for prostate cancer

The last several years have seen an increase in the number of clinical trials using vaccine immunotherapy for the treatment of prostate cancer. The trials have used a variety of target antigens that have been shown to be associated with prostate and prostate cancer cells. These include PSA (39,42-49), prostatic acid phosphatase (PAP) (50-53), prostate specific membrane antigen (PSMA) (54-56), telomerase (hTERT) (57,58), Thomsen-Friedenreich antigens (59), mucins (60), carbohydrates (61), and HLA-associated peptides (62). A variety of vectors have been used in the immunization process that include dendritic cells (45,50-58,63), vaccinia virus (39,42,43,47,49), fowlpox virus (39,47), liposomes (44), plasmids, (48), and chemical conjugates (59-61).

Recently, Kaufman et al. recently reported the results of a phase II trial (ECOG 7897) with a prime/boost vaccine using vaccinia virus and fowlpox virus expressing human PSA in patients with hormone-dependent prostate cancer (64). Sixty-four eligible patients with biochemical progression after local therapy were randomly assigned to three treatment arms: (A) fowlpox-PSA (rF-PSA) by intramuscular injection every six weeks for four doses, (B) rF-PSA for three doses followed by vaccinia-PSA (rV-PSA) given by intradermal injection, or (C) rV-PSA followed

by three rF-PSA vaccines. Dreicer et al. reported a randomized phase II study with a recombinant Modified Vaccinia Ankara virus which expresses both MUC1 and IL2 (TG4010) (65). MUC1 is a glycoprotein associated with several malignancies. Eligible patients were required to have no evidence of metastatic disease following curative intent local therapy, evidence of PSA failure (PSA over 2 ng/ml) and PSA doubling time (PSA-DT) less than 10 months. Arm 1 had TG4010 injected sc weekly, at a dose of 10^8 pfu, for six weeks, then every three weeks. Arm 2 had 10^8 pfu TG4010 injected sc every three weeks. Therapy was continued to disease progression or to a maximum of 36 weeks. Lastly, Small et al. recently presented the results of a phase III trial with APC8015, an immunotherapy cellular product consisting of autologous peripheral blood mononuclear cells enriched for a dendritic cell fraction pulsed with PA2024, a Prostatic Acid Phosphatase (PAP)-GM-CSF construct (66). Patients with asymptomatic, metastatic hormone-refractory prostate cancer were randomized (2:1) to receive APC8015 (n=82) or placebo (n=45) every 2 weeks x 3.

ECOG is currently planning a phase III trial using the Vaccinia virus (PROSTVAC-V/TRICOM) followed by Folwlpox virus vaccination (PROSTVAC-F/TRICOM) with GM-CSF, compared with placebo vaccine plus GM-CSF in patients with hormone-refractory prostate cancer with absence of metastatic disease (ECOG 1805, PARADIGM).

In summary, the results from these trials vary in terms of patient populations studied (hormone dependent vs. independent) and in levels of positive results, which include the induction of antigen-specific immune responses, decreases in levels of serum PSA and in rates of change in PSA velocity, and measures of clinical responses. Thus far no single vaccine immunotherapy has proven to be definitely superior to others in terms of clinical benefit, and other phase II and III trials continue to be planned or conducted. The results of some of these vaccine trials raise the question that an increase in PSADT may in the future represent a possible surrogate marker for increased time to progression, or overall survival in immunotherapy studies, and that absolute PSA responses may not constitute an obligatory step for the ultimate demonstration of clinical benefit of immunotherapy approaches in prostate cancer. Furthermore, the T-cell stimulation index may have important correlation with clinical vaccine efficacy, as seen in the phase III trial by Small et al.(66). These developing notions further support the current proposal for clinical development of our Ad/PSA vaccine, also based on the results of our prior phase I trial.

1.6 Proposed phase II clinical trial: rationale

Based on the significant pre-clinical activity of the Ad/PSA vaccine in generating tumor-specific T cells, and the encouraging safety and efficacy results from our phase I study, we propose to continue the clinical development of the Ad/PSA vaccine with the performance of the current phase II trial. It is our contention that the vaccine product and the method of immunization set this therapy apart from other ongoing prostate cancer investigational immunotherapeutic approaches. Specifically, the incorporation of Gelfoam, not present in other vaccine preparations, enhances the induction of strong anti-PSA responses. Immunization of mice with Ad/PSA in Gelfoam matrix was able to induce anti-PSA responses even in the presence of high-titer anti-adenovirus antibodies. Notably, most humans naturally possess high titers of anti-adenovirus antibodies due to natural exposure to adenoviruses.

We plan to enroll prostate cancer patients into one of two arms (A & B) of the Phase II clinical trial. The ideal patient population to determine a therapeutic benefit of a new treatment, particularly immunotherapy, is one with minimal disease burden. The low tumor burden should allow therapies, particularly those relying on antigen-specific effector T lymphocytes, to destroy

all of the cancerous tissues and cells. The first therapeutic arm (Arm A) will enroll men with recent evidence of recurrence following surgery or radiation therapy for their primary tumor. Patients in the population will be eligible if they exhibit at least four separate rises in serum PSA, at least one month apart with differences $\geq$0.03 ng/ml and a total PSA of >0.2 ng/ml; have a PSA doubling time of $\geq$6 months; not at high risk or patients that refuse treatment with Taxotere or radiation. A high risk patient will be defined as those with a serum PSA of >20 ng/ml and a Gleason score of >7. All patients will be hormone naïve. Since standard therapy for these patients would be to postpone androgen ablation therapy until such time as there is a high serum PSA level ($\geq$20 ng/ml), enrolling patients into this Phase II trial does not withhold accepted treatment. Patients will be excluded from the trial if they are candidates for salvage radiation therapy, had multiple positive margins at surgery, had a serum PSA of >20 ng/ml prior to surgery, a Gleason score of >7, seminal vesicle involvement or positive lymph nodes.

The second therapeutic arm (Arm B) will enroll men with recurrent disease who will undergo androgen depletion therapy. The choice of this additional patient population is based upon published documentation that inflammation and the generation of immune responses are augmented by hormone withdrawal (67-69). Mercader, et al., in attempts to demonstrate an enhanced termination of tolerance to prostate associated antigens documented CD4+ and CD8+ T cell infiltrates in benign prostates and in prostate tumors of men undergoing androgen withdrawal (67). Roden and co-workers published data demonstrating that T cell levels and T cell proliferation were increased in mice following castration (68) while Drake, et al. reported breaking tolerance to antigens associated with the TRAMP prostate tumors in mice (69). Therefore, we propose to vaccinate men beginning 14 days after the initiation of androgen depletion therapy using the same three injection protocol.

As part of consideration for enrollment, the study will only enroll subjects for whom androgen depletion therapy or deferral of such treatment are considered standard of care options. In addition, in order to be considered for the study, patients must be willing to be in either study group (Arm A or Arm B). Randomization of patients to Arm A (vaccine only) or Arm B (ADT plus vaccine) will be 1:1. Fifty (50) patients will be enrolled, 25 in each Arm of the study. Twenty five (25) cards will be printed for Arm A and 25 cards printed for Arm B and all 50 cards will be placed in a container. Following the determination of eligibility of each patient and administration of the Informed Consent document, a card will be drawn to determine which Arm of the study the patent is entered.

We will enroll and treat patients at two affiliated and adjacent medical centers, the University of Iowa Hospitals and Clinics (UIHC) and the Iowa City Veterans Affairs Medical Center (ICVAMC). There is a single Institutional Review Board (IRB) that approves protocols for both institutions. After the University of Iowa's IRB has approved the protocols the Research and Development Committee of the ICVAMC meets to provide their approval for the study.

Patient Recruitment: The UIHC and ICVAMC draw from a large catchment area that will insure the enrollment of sufficient numbers of men into the protocol. We will use recruitment methods similar to the methods used to obtain subjects for our Phase I clinical trial. That includes contacting patients that are being or have been treated in the Departments of Urology, Internal Medicine, and Radiation Oncology at the UIHC and ICVAMC and letters written to urologists, oncologists, and medical oncologists in Iowa and the neighboring states of Wisconsin, Illinois, Missouri, Nebraska, Minnesota, and South Dakota. Drs. Williams and Joudi will assist in the recruitment of patients from the Department of Urology, Dr. Vaena from Internal Medicine, and Dr. Smith from Radiation Oncology.

A physician from outside the UIHC or ICVAMC may inform patients of the study. If a patient expresses interest in learning more about the trial, the physician will provide him with contact information for the clinical trial team. When a patient contacts the clinical trial team, a member of the trial team will briefly describe the study and request the patient's mailing address in order to mail him a copy of the study informed consent form.

After the patient has had time to become familiar with the study and discuss it with his family, a member of the research team will follow up with a telephone call to discuss the study and answer any questions the patient may have. Of particular importance is the patient's understanding that he will be assigned to one of two arms in the protocol, vaccine alone or androgen deprivation therapy followed by vaccine. At this time, if the patient is still interested in participating, he will be asked to provide verbal consent to allow the research team to review information from his medical record to evaluate his eligibility for the trial. The researcher will document the verbal consent process in the research file, and will contact the patient's physician to request the medical record for review. The patient will be required to sign a HIPAA privacy form and a Release of Medical Information form at his physician's office prior to the physician releasing his records to the research team.

After reviewing the relevant portions of the medical record, a member of the research team will again contact the patient. Eligible individuals will be scheduled for a visit to the UIHC or ICVAMC to undergo additional eligibility testing. At that visit, informed consent for participation in the clinical trial will be obtained. The subject will then be further evaluated for study eligibility through a medical history and physical exam, blood tests, and scans. Final determination of eligibility will occur at a weekly meeting of the clinical trial team.

2. **OBJECTIVES**

2.1 Primary Objective

To evaluate the development of anti-PSA immune responses in study patients, of particular importance is the comparison of immune responses generated in the hormone naïve and ADT patients.

2.2 Secondary objectives

2.2.1 To evaluate the response rates (PSA responses and changes in PSADT) of the Ad/PSA vaccine using a prime-boost immunization strategy, in patients with recurrent disease, either hormone naïve or during androgen deprivation therapy (ADT).

2.2.2 To evaluate biochemical (PSA recurrence) and radiographic (bone scans) time to progression and overall survival in evaluable patients receiving the Ad/PSA vaccine.

3. **SELECTION OF PATIENTS**

As described in Section 1.6 prostate cancer patients will be enrolled in one of two arms of the study. All men will have recurrent disease and who are initially hormone naïve, having received no prior hormonal therapy. Following the determination of eligibility to participate in the protocol, one group of patients will receive the Ad/PSA vaccine alone (Arm A) and a second

group of patients will begin androgen deprivation therapy with vaccination beginning fourteen days later (Arm B).

3.1 Inclusion criteria:

3.1.1 Men with prostate cancer who have received prior local therapy (radical prostatectomy or definitive radiation therapy) and have biochemical (PSA) relapse without evidence of radiographic or clinical metastatic disease.

3.1.2 For men who had prior prostatectomy, the surgery must have occurred at least 6 months prior to study enrollment.

3.1.3 For men who had prior definitive radiation therapy, radiation must have occurred at least 1 year prior to study enrollment.

3.1.4 Exhibit at least four separate rises in serum PSA, at least one month apart with differences $\geq$0.03 ng/ml and a total PSA of >0.2 ng/ml.

3.1.5 Have a PSA doubling time of $\geq$6 months.

3.1.6 Not at high risk as defined as those with a serum PSA of >20 ng/ml and a Gleason score of >7 before prostatectomy or radiation.

3.1.7 Negative bone scans.

3.1.8 Negative CT scans of chest abdomen and pelvis (no soft tissue metastases present).

3.1.9 Scans must be obtained within 6 weeks of entry into the trial.

3.1.10 Written informed consent.

3.1.11 Age $\geq$ 18 years.

3.1.12 Required laboratory values (obtained within 2 weeks of study entry)

3.1.12.1 Serum creatinine $\leq$ 2.0 mg/dL

3.1.12.2 Adequate hematologic function: granulocytes $\geq$ 1800 per mm^3, platelets $\geq$ 100,000 per mm^3, WBC $\geq$3700, and lymphocytes $\geq$590.

3.1.12.3 Adequate hepatocellular function: AST <3x normal and bilirubin <1.5 mg/dl.

3.2 Exclusion criteria:

3.2.1 Candidates for salvage radiation therapy unless the patient refuses.

3.2.2 Had a serum PSA of >20 ng/ml prior to surgery or radiation.

3.2.3 Gleason score of >7.

3.2.4 Seminal vesicle involvement or positive lymph nodes.

3.2.5 Active or unresolved infection.

3.2.6 Parenteral antibiotics <7 days prior to study entry.

3.2.7 Evidence of prior or current CNS metastases. Specific imaging is not necessary in the absence of signs or symptoms.

3.2.8 Co-morbid medical conditions which would result in a life expectancy (participation) of less than 1 year.

3.2.9 Patients with compromised immune systems; congenital, acquired, or drug-induced (immunosuppressive agents) will be excluded from the study. Use of prednisone at doses higher than 10 mg daily (or equipotent steroid doses) for more than 7 days within the last 3 months is not allowed.

3.2.10 No-pre-existing malignancies that required treatment within the past 5 years except for basal or squamous cell cancers of the skin.

3.2.11 Prior systemic therapies for prostate cancer not allowed (hormonal therapy, including but not limited to LHRH agonists, antiandrogens, ketoconazole or chemotherapy – mitoxantrone/taxanes/estramustine, etc.); only patients in Arm B, undergoing androgen depletion therapy during the vaccination will be eligible.

3.2.12 Prior participation in any vaccine studies for any disease.

3.2.13 The inability to understand the language and the clinical protocol.

3.2.14 Allergy or religious objection to pork products; Gelfoam is produced from pork.

4. Registration Procedures

4.1 All patients will be registered through the Department of Urology at the University of Iowa Hospitals and Clinics (UIHC) or the Urology Service at the Iowa City Veterans Affairs Medical Center (ICVAMC).

4.2 Patients who are candidates for enrollment into the study will be evaluated for eligibility by the clinical investigators to ensure that the criteria outlined in Section 3 have been satisfied and that the patient is eligible for participation in this clinical investigation. The University of Iowa will provide a patient eligibility case report form for this evaluation.

4.3 Following determination of eligibility patients will be assigned to receive the vaccine alone (Arm A) or ADT followed by the vaccine (Arm B).

4.4 Informed Consent - Signed informed consent for enrollment in this protocol will be obtained from eligible patients by the attending physician, study coordinator, or clinical trial coordinator before the start of the research intervention. At the preadmission consultation, patients will be fully informed of the purpose and potential risks and benefits of participating

in the study. Patients have the opportunity to have questions answered to their satisfaction before signing the consent.

4.5 Eligible patients must be registered Monday through Friday between 8:00 a.m. and 4:30 p.m. (Central Time) by calling Carlene Etscheidt, BSN, MSN or Pamela Zehr, BSN, MA the University of Iowa Clinical Cancer Center, Iowa City, Iowa, 319-356-1228 or 319-353-8914 respectively. Patients at the ICVAMC will be registered by calling Sara Miller, BSN at 319-338-0581, ext. 7519. Information from the eligibility form will be provided by the investigator or the investigator's research staff to the University of Iowa Cancer Center at this time, and the patient will be registered and assigned a unique patient number.

4.6 No patient may be enrolled or begin the research intervention prior to registration and assignment of a patient number. As a follow-up, University of Iowa Cancer Center will provide the investigator with written confirmation of each patient's registration.

4.7 All investigators will be notified by the Chair of the Protocol Review and Monitoring Committee or by the trial's Data and Safety Monitoring Board if the study is placed on administrative hold, and when the study is completed or closed to further patient enrollment.

4.8 Patients must begin the vaccine protocol, either the vaccine alone (Arm A) or begin ADT (Arm B) followed within 7 days of registration.

5. RESEARCH INTERVENTION PLAN

5.1 Administration Schedule

Ad/PSA

Patients with recurrent disease after surgical treatment or radiation therapy will be randomized to either Arm A (vaccine only) or Arm B (androgen depletion therapy plus vaccine). All patients will receive three injections of 0.125 ml. of the Ad/PSA subcutaneously in the right thigh. The dose of the vaccine, based upon our results from the Phase I trial, will be 1×10^8 pfu (4.4×10^9 particles) in the Gelfoam matrix. The Gelfoam comes in sterile patient-ready packages. The virus will be suspended in sterile saline and the Gelfoam powder added in a ratio of 30 mg of powder per ml. of virus suspension. Injections will be spaced apart by 30 days, such that each patient will receive the vaccine on days 0, 30, and 60. The use of the matrix has been shown in collaborative pre-clinical experiments to enhance infection of host cells by the virus. Results from the Phase I trial indicated that the injection of the vaccine in Gelfoam did not produce any adverse events greater than those produced by the vaccine in an aqueous suspension. The vaccine induced anti-PSA immune response in patients injected as an aqueous or Gelfoam vaccination. Injections will be carried out in the University of Iowa General Clinical Research Center (GCRC). Each subject will be housed in the GCRC for 24 hours and observed for early signs of toxicities following each of the three vaccinations. Tests, indicated in the table on page 23, will be carried out to be certain that no serious side effects are induced by the vaccine.

5.2 Design and Stages

Subjects will enter the study according to a phase II two-stage design Simon (1989). We anticipate a total of 25 subjects (per arm) if the stopping rules do not signal futility. After testing the research intervention on 10 patients, if 3 did not have the desired increase in

anti-PSA T cells, the trial will be terminated. Otherwise, an additional 15 patients will be allowed to enter the trial for a total of 25 patients with the goal that more than 13 will show the desired increase. For both arms, we will need 50 subjects. For statistical considerations and the power reached under this study design consideration, we refer the reader to Section 10.

6. Adverse Events

Toxicity will be graded according to the NCI common toxicity criteria "Common Terminology Criteria for Adverse Events" (NCI-CTCAE v3.0 can be accessed at website: http://ctep.cancer.gov/forms/CTCAE_Index.pdf). Non-hematologic dose limiting toxicity (DLT) will be defined as grade III non-hematologic toxicity. Hematologic DLT will be declared if patients develop grade IV or grade III and fail to recover their absolute neutrophil count (ANC) and platelets to Grade I/II levels after 5 weeks, unless such failure is due to progressive tumor.

6.1 Definitions

Adverse Event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this research intervention. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease* temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

This will also include intercurrent diseases and accidents observed during the research intervention period as well as corresponding events during drug-free, pre- and post-intervention periods, under placebo or in a reference group receiving drug or non-drug therapy.

Serious adverse event (SAE) is any untoward medical occurrence that:

a. results in death
b. is life-threatening[i]
c. requires inpatient hospitalization or prolongation of existing hospitalization
d. results in persistent or significant disability or incapacity
e. is a congenital anomaly / birth defect or
f. is another medically important condition.[ii]

6.2 Procedures of documentation of AEs

All AEs occurring during the study must be documented, regardless of the assumption of a causal relationship, on the respective AE CRF. All events, which occurred after signed informed consent, should be documented. The investigator should ensure that all events are recorded that occurred within at least 4 weeks after the last exposure to the study drug.

Documentation of AEs includes: date of onset and offset, intensity, frequency, seriousness, related interventions and outcome. The investigator will also evaluate the

probability of a causal relationship of the adverse event to the study medication as being: “definite, probable, possible, unlikely, or unrelated.”

All unanticipated problems involving risk to subjects or others, serious adverse events related to participation in the study and subject deaths related to participation in the study will be promptly reported by phone (301-619-2165), by email (hsrrb@det.amedd.army.mil), or by facsimile (301-619-7803) to the USAMRMC, Office of Research Protections, Human Research Protection Office. A complete written report will follow the initial notification. In addition to the methods above, the complete report will be sent to the U.S. Army Medical Research and Materiel Command, ATTN: MCMR-ZB-PH, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

The medical monitor is required to review all unanticipated problems involving risk to volunteers or others, serious adverse events and all volunteer deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the medical monitor should comment on the outcomes of the event or problem, and in the case of a serious adverse event or death, comment on the relationship to participation in the study. The medical monitor should also indicate whether he/she concurs with the details of the report provided by the study investigator. Reports for events determined by either the investigator or medical monitor to be possibly or definitely related to participation and reports of events resulting in death should be promptly forwarded to the HSRRB.

Expedited reporting

The investigator must immediately report serious adverse events (SAE) occurring or observed during the course of the study and within 4 weeks of last administration of the study drug to the FDA, IRB, OBA, and GCRC.

After notifying the proper agencies by telephone of an SAE within 24 hours of the knowledge of the event’s occurrence, the “Serious Adverse Event Report ”must also be sent by fax to the agencies whether or not complete information is available at the time. If complete information is unavailable the investigator must provide follow-up information to the agencies as soon as it is known.

In particular, the investigator must inform the agencies by phone and fax within 24 hours of occurrence of immediately life-threatening SAEs or SAEs with fatal outcome. SAEs must be reported to the site’s IRB according to the IRB’s requirements.

Important: The investigator must report any SAE to the FDA, IRB, OBA, and GCRC, regardless of causality.

Reports will be evaluated by the Medical Monitor/Sponsor. FDA/HPB and investigators will be informed as required by the regulations. The same information will also be made available to all participating investigators as well as to other investigators participating in different clinical trials utilizing the same study medication.

It should be noted that, although the Ad/PSA vaccine contains the gene for PSA, there is no need for patients to utilize contraceptive practices. The adenovirus will not be transmitted from patient to his partner.

In addition, the side effects of androgen depletion therapy, fluid retention, hair loss, hot flashes, nausea, vomiting, bone pain, memory changes, and depression, were not observed in our Phase I trial. Therefore, there should not be any confusion as to which side effects will be attributed to ADT and which to the Ad/PSA vaccine. However, subjects will be closely monitored by the research team for the development of ADT-

related side effects. If side effects occur, investigators will take necessary steps to treat them, including discontinuation of the medication and/or discontinuation from the study.

7 MEASUREMENT OF CLINICAL AND IMMUNOLOGICAL EFFECT

7.1 Methods of Malignant Disease Evaluation - Each patient will have a baseline evaluation prior to the injection of the Ad/PSA vaccine. The measurements will include temperature, weight, serum PSA, blood chemistries, a quantitative bone scan for bone metastases, chest x-ray, and CT for soft tissue metastases, and performance status for quality of life. PSA measurements, CT and bone scans are routinely used to follow disease recurrence and/or progression in individual prostate cancer patients and as such would be considered standard of care. Laboratory measurements such as hematology, liver function and kidney function chemistries, while are routinely used to follow the health of a prostate cancer patient, would not normally be performed at the frequency proposed in this trial to assess possible vaccination toxicities. Therefore, they would be considered part of the research protocol.

Patients will be seen in the GCRC (see Table 6 for schedule). The injection site will be examined for evidence of erythema, induration and necrosis and patients will have their temperature and weight recorded and interviewed to determine whether they experienced any adverse reactions. Blood samples will also be taken for measurement of PSA and anti-PSA antibodies (see Table 6). At the 6 month, 12 month, and subsequent semi-annual visits each patient will be evaluated using the measurements listed for the baseline visit.

Because changes to the clinical status of the patients will be prolonged, long-term follow up will be important. Therefore, patients will return to the clinic every six months following the 12 month post-vaccination visit. The visits will continue indefinitely unless the patient demonstrates signs of progressive disease.

7.2 Use of Serum PSA for Disease Evaluation – Based upon our pre-clinical experiments and the results from the Phase I clinical trial we expect the immunized men to produce anti-PSA antibodies. The levels of antibody will be measured by a flow cytometry assay as described by Cavacini, et al. used in our Phase I clinical trial (39). We will also explore the use of a second serum marker for prostate cancer, hK2 in collaboration with Donald Tindall, Charles Young, and George Clee at the Mayo Clinic. Investigators at Mayo, along with Hybritech, Inc. have been exploring hK2 and published a number of papers in recent years on the subject (70-73). Patient sera from each clinic visit will be sent to Mayo where they will measure the levels of hK2. We will use the data to evaluate the effect of anti-PSA antibodies on both PSA and hK2 in the sera of vaccinated patients.

7.3 Experimental Evaluation of the Ad/PSA Vaccination

7.3.1 Blood will be collected prior to, and at each visit after, the injection of the Ad/PSA vaccine. Two separate samples will be collected; one in red top tubes to allow collection of serum from coagulated blood and a second in heparinized tubes to permit collection of lymphocytes.

7.3.2 Levels of PSA, hK2, anti-PSA antibodies, and anti-adenovirus antibodies will be measured in the serum.

7.3.3 Anti-PSA T cell immune responses will be measured by ELISPOT analysis using the methods developed for, and used in, our Phase I clinical trial. In addition to measuring the anti-PSA T cell activity, we will also measure anti-adenovirus T cell activity as well as reactivity to stimulation with cytomegalovirus (CMV). A non-specific stimulus will be provided by PMA and ionomycin for each patient's lymphocytes.

7.4 Definitions of Response –

7.4.1 Arm A – Hormone Naïve –

7.4.1.1 Primary Endpoint - Development of Anti-PSA Immune Responses

7.4.1.1.1 Immunologic response – Definition: an increase of ≥200% above re-immunization levels of anti-PSA T cells as measured by ELISPOT analysis, measured at any point after vaccination.

7.4.1.2 Secondary Endpoints – PSA Responses

7.4.1.2.1 PSA response - Definition: a 50% reduction in the pre-research intervention PSA value, verified with a second measurement 30 days later

7.4.1.2.2 A 50% increase in the PSADT compared to pre-enrollment PSADT.

7.4.1.2.3 PSADT will be calculated based on the MSKCC online calculator, available at http://www.mskcc.org/mskcc/html/10088.cfm.

7.4.1.2.4 PSADT response will be measured at 9 and 18 months after initiation of study the research intervention.

7.4.1.2.5 Three measurements of PSA, spaced at least 2 weeks apart, will be required prior to study enrollment. Post-research intervention PSADT will be based on PSA levels at 3, 6 and 9 months (9 month PSADT calculation) and 3,6, 9,12,15,18 month levels (18 month PSADT calculation).

7.4.2 Arm B – Androgen-Deprivation Therapy –

7.4.2.1 Primary Endpoint - Immunologic response

7.4.2.1.1 Definition: an increase of ≥200% above re-immunization levels of anti-PSA T cells as measured by ELISPOT analysis, measured at any point after vaccination.

7.4.2.2 Secondary Endpoint - PSA response - Definition: a 50% reduction in the pre-research intervention PSA value, verified with a second measurement 30 days later

7.5 Definition of Progression

7.5.1 Development of positive bone scan (bone scans will be performed every 6 months)[B]

7.5.2 Development of rising PSA after nadir, if existent

7.6 Timing of Toxicity Assessments - Toxicity assessment will occur as stated in the calendar. We will wait for the last patient of stage I to reach the 90 day toxicity assessment date (and review of safety data) prior to proceeding with stage II as outlined in the statistical plan.

8 STUDY PARAMETERS

8.1 Scans or x-rays used to document measurable or evaluable disease should be done with 4 weeks prior to study entry

8.2 CBC with differential, LFT's should be done ≤2 weeks before study entry.

8.3 All chemistries should be done ≤2 weeks before the study entry, unless specifically required on day 1 as per protocol. If abnormal, they must be repeated within 48 hours prior to study entry.

8.4 Hgb, Hct, WBC, Plt should be done ≤2 weeks before study entry but, if abnormal, they must be repeated <48 hours prior to study entry.

8.5 REMOVAL OF PATIENTS FROM STUDY (Criteria for discontinuation of a patient's study participation)

8.5.1 Adverse events: In the event of a vaccine-associated unmanageable or irreversible toxicity, that would include hematological and non-hematological toxicities, the investigator will withdraw a patient from further research intervention and notify the Study Chair immediately. *In addition, the FDA and the IRB will be notified of the adverse events.* If unmanageable or irreversible toxicities occur the patients will receive the best possible medical care according to the recommendations of the treating physicians. The treatment plan will depend upon what unmanageable or irreversible toxicities may occur. In the event of an emergency the patient will be instructed to seek immediate care by calling 911, his local physician, the University of Iowa or Iowa City VA Medical Center urology staff on call.

8.5.1.1 Management of Toxicities

8.5.1.1.1 Hematological Toxicity Management

8.5.1.1.1.1 Patients with grade 2 or 3 hematological toxicity will not receive the subsequent dose of vaccine until there is normalization of cbc parameters, as required per eligibility criteria.

[B] Only patients with negative bone scans are eligible for the study

8.5.1.1.1.2 In case of grade 2 or 3 hematological toxicity detected any time during protocol therapy, CBC with differential will be checked on a weekly basis.

8.5.1.1.1.3 If the subsequent vaccine dose needs to be postponed for more than 2 weeks, patients will be removed from protocol therapy.

8.5.1.1.1.4 Patients with grade 4 hematological toxicity at any time will be permanently removed from protocol therapy.

8.5.1.1.2 Non-Hematological Toxicity Management

8.5.1.1.2.1 Patients with grade 2 or 3 non-hematological toxicity will not have the subsequent dose of vaccine administered until the toxicity ameliorates down to a grade 1 level.

8.5.1.1.2.2 Patients will be seen twice a week with study physician visits, if a grade 3 non-hematological toxicity occurs.

8.5.1.1.2.3 If the subsequent dose of vaccine needs to be postponed for more than 2 weeks, patients will be permanently removed from protocol therapy.

8.5.1.1.2.4 Patients with grade 4 non-hematological toxicity at any time will be permanently removed from protocol therapy.

Patients may also be removed from protocol therapy at any time based on assessment of any other risks, at the discretion of the study investigators.

8.5.2 Disease Progression: Patients will be taken off-study if they have progressive disease (PD) or clinically significant deterioration at any time during the study if the investigator feels that (a) alternative prostate cancer therapy might benefit the patient, or (b) to continue on study might be unsafe for the patient. Patients receiving alternative prostate cancer therapies will still be followed for toxicity and immunologic evaluations.

8.5.3 Allergic Reactions: Patients will be removed from the study should they develop grade II allergic reactions.

8.5.4 Personal Reasons: As stated in the informed consent, patients may withdraw from the study at any time.

8.5.5 Clinical Judgment: A patient may be withdrawn from the study, if, in the opinion of the investigators, it is not in the patient's best interest to continue (e.g. an adverse experience, intercurrent illness, etc.)

8.5.6 The date of discontinuation and the reason(s) for patient discontinuation from the study will be recorded in the CRF. All evaluations that are required at the follow-up must be conducted for each patient who discontinues research intervention, regardless of the reason.

Regulatory and Reporting Requirements

The Data and Safety Monitoring Committee (DSMC) of the Holden Comprehensive Cancer Center will provide data and safety monitoring for this study. “The Data and Safety Monitoring Plan of the Holden Comprehensive Cancer Center” provides standard operating procedures to monitor all clinical cancer trials at the UIHC. All investigator-initiated trials are automatically monitored by the DSMC. A detailed data and safety monitoring plan for this study is on file with the DSMC and the Clinical Research Safety Officer (CRSO).

Data Management, Quality Control and Data Security

In order to protect confidentiality the subject will be assigned an identification number. This number will be used on all specimens from the subject and will be used for documentation purposes.

Data management for the optimal entry, processing, storage, and retrieval for this protocol's data will be accomplished by the principal investigator. The database will be located on a computer or in a locked cabinet in a locked office. This computer will be secured, accessible only by the research team. There will be more than one copy of the database. The second, secured, copy of the protocol data will be stored in a locked room accessible only by the research team. For quality control, auditing, and checking data for integrity, there will be a regular accounting of data performed. The medical record and research record will be linked by the study identification number. The data managers in this trial will be responsible for verification of the accuracy of all data transferred from the medical record to the research record. These records will be audited quarterly by the Data Safety Monitoring Committee. All Data Safety Monitoring Committee reports will be provided to the USAMRMC Office of Research Protections, Human Research Protection Office as they become available.

Data will be kept on file on each patient for at least two years past the termination of that patient’s participation in the trial. After that period of time the data in the research records will be shredded and the electronic database deleted.

Information from the medical records of patients referred by physicians outside the University of Iowa and the VA Medical Center required to determine eligibility for this protocol will be placed in a research folder, identified by the patient code and will be available only to members of the clinical trial team (identified on pages 28 and 29). The signed HIPAA forms will be kept in the patient’s folder in the office of the referring physician. The records of such patients that are deemed ineligible following a screening procedure will have their signed Release of Information forms, informed consent document, and eligibility form kept in a research folder under similar security conditions. All other records for these ineligible patients will be shredded unless the information is required for required ongoing medical care.

Table 6
Study Design and Testing

	Std. of Care or Res.	Prior to Study Entry	Day 1 First Inj.	After first 24 hrs.	30 d. 2nd Inj.	31 d.	44 d.	60 d. 3rd Inj.	61 d.	74 d.	90 d.	6 mo	9 mo	12 mo	Every 6 mo. to prog.	Annual to prog.
Immunization	R		X		X			X								
Physical Examination	S	X	X	X	X	X		X	X		X	X	X	X	X	
Performance Status	S	X			X			X			X	X	X	X	X	
Vital Signs	S	X	X	X	X	X		X	X		X	X	X	X	X	
Weight	S	X	X		X			X			X	X	X	X	X	
Blood for PSA	S/R	X			X (R)			X (R)			X	X	X	X	X	
anti-PSA Ab, and lymphocytes for cellular immunity	R	X	X		X		X	X		X	X	X	X	X	X	
CBC, differential	R	X		X	X			X			X	X	X	X	X	
AST, ALT, LDH, alkaline phosphatase, bilirubin	R	X		X	X			X			X					
Creatinine	R	X		X	X			X			X					
Urinalysis	R	X		X	X			X			X					
Chest x-ray	R	X[a]			X[a]			X[a]			X[a]	X[a]	X[a]	X[a]	X[a]	
Bone scan	S	X[b]						X[b]				X[b]		X[b]		X[b]
Abdominal/pelvic CT	S	X[c]										X[c]		X[c]		X[c]
Serum testosterone			X		X			X								

S = Procedures considered “Standard of Care” for prostate cancer patients.
R = Procedures considered “Research” as part of this Clinical Trial.

60 ml. of blood will be obtained at each time point for all clinical and research testing.

[a] will be repeated only if abnormal at screening or if patient develops a fever.
[b] will be repeated only if he patient demonstrates a rise in PSA
[c] will be repeated only if abnormal at screening.

9 DRUG FORMULATION AND PROCUREMENT

9.1 Drug Name

Adenovirus/PSA (Ad/PSA)

9.2 Classification

Vaccine

9.3 Mode of Action

The adenovirus is a replication-deficient virus unable to produce virus progeny in the infected cells. The virus will infect cells in the location of the injection site, the PSA gene will produce the protein product which will be recognized as an antigen by the immune system and produce anti-PSA immune responses. Based upon our pre-clinical studies in an animal model of human prostate cancer, these responses, mainly the CD8+ CTL response, will cause the destruction of PSA-secreting prostate tumors.

9.4 Dose Specifics and Route of Administration

The route of injection, vehicles for the vaccine, and dose schedules have been outlined in Section 5.1 of this protocol.

9.5 Availability

Produced and provided by Molecular Medicine, LLC, San Diego, CA

9.6 Storage

The vaccine is stored in the UIHC's Pharmacy Department in a -70^{o}C temperature-monitored and controlled access freezer. Only the Investigational Pharmacist will remove the vaccine from the freezer and enter the amount removed in a trial-specific log.

9.7 Injection Procedures & Twenty-Four Hour Observation

At the weekly meetings of the clinical trial team at which the eligibility of the patients is decided, orders for the initial vaccine injection will be written by clinician members of the team. Orders for the second and third vaccine injections will be written at the meetings immediately prior to the date of the scheduled injections. On the designated days of injections and immediately prior to vaccination, the Investigational Pharmacist will mix the Ad/PSA (10^8 pfu or 4.4×10^9 particles) with the Gelfoam powder (30 mg/ml). Each vial of the vaccine contains twice the volume needed for each injection. One half of the vial will contain the proper pfu and particles required for the vaccination. The injection material will be taken from the pharmacy in a 1 ml. syringe with 25 gauge needle and presented to staff in the General Clinical Research Center. Each patient will be injected with approximately 0.125 ml. of the vaccine/Gelfoam material subcutaneously in the thigh. The exact volume of the vaccination mixture (Ad/PSA in Gelfoam) is not as important as the exact number of pfu or particles and that is

controlled by using the required volume from the dose vial. The empty syringe will be disposed of in a biohazard container.

During the 24 hour stay in the GCRC the staff in the clinic will monitor patients for signs of adverse events. Physical examinations, vital signs, and laboratory tests will be performed as delineated in Table 6 on page 23 will be taken. Based upon the adverse events observed in the Phase I study the GCRC staff will look for signs of inflammation at the injection site, fever, cold and flu-like symptoms, fatigue, and changes in the absolute neutrophil count (ANC). The decision to discharge the patient at the end of the 24 hour period will be responsibility of one of the physician investigators, Drs. Williams, Joudi, Vaena or Smith.

9.8 Assignment of Patients to Arms A or B

Randomization of patients to Arm A (vaccine only) or Arm B (ADT plus vaccine) will be 1:1. Fifty (50) patients will be enrolled, 25 in each Arm of the study. Twenty five (25) cards will in printed for Arm A and 25 cards printed for Arm B and all 50 cards will be placed in a container. Following the administration of the Informed Consent document and determination of eligibility of each patient, a card will be drawn to determine which Arm of the study the patent is entered. Patients that choose not to be placed on ADT will not be assigned to the Arm B treatment group. For this study, ADT will consist of LHRH or GNRH agonists, such as leuprolide (Lupron) or goserelin (Zoladex). Anti-androgens or aromatase inhibitors will not be used. Patients in Arm B will be vaccinated 14 days after the initiation of ADT. To insure that all patients reach castrate levels of testosterone (<5 ng/ml), serum T levels will be measured on their day 30 visit, prior to their second vaccination and their 60 day visit, prior to their third vaccination. These times will be approximately 6 and 10 weeks after the initiation of androgen withdrawal, a time at which T levels should reach their nadir.

9.9 Manufacturing

9.9.1 The PSA cDNA provided by Donald Tindall, Mayo Clinic, Rochester, MN, was placed 3' to the CMV promoter in a shuttle vector containing Ad5 DNA. The sequence inserted was the pre-pro form of PSA described by Lundwall (75) that encodes 262 amino acids with a predicted molecular weight of 28.8 kDa. Using methods previously described (75), the shuttle vector and E1a-E1b deletion mutant Ad5 DNA were transfected into HEK 293 cells, and recombination between the DNA species was allowed to occur. The amplification and purification of Ad/PSA was performed by the University of Iowa Gene Transfer Vector Core as previously described (76). Ad/lacZ used as a control was also obtained from the Gene Transfer Vector Core and is previously described (75).

9.9.2 The Principal Investigator provided the Ad/PSA vaccine used for the pre-clinical studies to Molecular Medicine, LLC of San Diego, CA for the production of the clinical grade product. Information on the manufacturing of the GMP Vaccine by Molecular Medicine, LLC is found in the accompanying documents supplied by the company.

10 STATISTICAL CONSIDERATIONS

The ideal endpoint would be a clinical outcome that is of particular relevance to the patient such as increased time to tumor progression, increased time to death, or reducing the

proportion of death. The trial is using a surrogate endpoint as a substitute to the clinically meaningful outcome since the tumor cannot be accessed directly. The association between the surrogate and survival rate had not been clearly established by any phase I & II trial. The trial consists of using Ad/PSA vaccine administered in multiple injections to prostate cancer patients with minimal disease burden—with the goal to induce anti-PSA T cells responses. Three injections of equal dose are proposed. A previous phase I trial consisting of a single injection in men with metastatic prostate cancer was able to induce anti-PSA T cells responses. The Phase I consisting of a single injection using a dose escalation protocol of the vaccine in an aqueous or matrix delivery vehicle did not show any significant AE. Additional pre-clinical pharmacology /toxicology studies required by the FDA did not show any significant side effects using the three-injection schedule. The primary endpoint is the development of anti-PSA immune response—defined as an increase of greater than 200% above re-immunization level of anti-PSA T cells as measured by ELISPOT analysis, measured 14 days after the 3rd immunization. This endpoint will be the same for both arms considered in the protocol—the vaccine only and the vaccine plus androgen deprivation therapy arms.

During the Phase I trial consisting of vaccine alone, patients that developed anti-PSA T cell responses had an increase in the frequency of anti-PSA T cells. We decided to express the anti-T cell response as percent change in antigen-specific T cell frequency compared to the frequency prior to immunization. Based on our phase I results, we judge 70% of the patients developing anti-PSA immune response as being clinically important while anything less than 40% in both arms will be judged unworthy.

The ideal design would be a two-stage design of Simon (1989). After testing the research intervention on 10 patients, if 3 did not have the desired increase in anti-PSA T cells, the trial will be terminated. Otherwise, an additional 15 patients will be recruited for the trial for total of 25 patients with the goal that more than 13 will show the desired increase. If 8 or more show the desired increase, then the research intervention will be judged worthy for further considerations. In case the number is less than 8, the research intervention will be rejected.

The expected sample size will be 15.14 and the probability of early termination, 0.63. If the research intervention is not effective, there is 0.05 probability of concluding that it is. If the research intervention is effective, there will be 0.09 probability of concluding that it is not.

We will be conservative and use the same design and sample size considerations for both arms (25 per arm for a total of 50 patients) even though we expect the vaccine plus androgen deprivation therapy arm to yield better results. This brings a natural question of why two different research interventions and how one would assess the superiority of one research intervention over the other. We postulate that men in Arm B, those receiving ADT plus vaccine, will generate a stronger anti-PSA immune response, as measured by the production of PSA-specific T cells, than will men in Arm A, those receiving the vaccine alone. For patients in Arm B, there will be a quantitative and/or qualitative difference in the generation of anti-PSA T cells. That is, a higher percentage of men will demonstrate antigen specific T cells and/or the magnitude of the T cell response, as indicated by the precursor frequency, will be greater in this population. The differences will be most evident 14 days after the third and last vaccination. A test for comparison of these two proportions will reveal the superiority of ADT plus Vaccine over Vaccine alone.

The secondary endpoint is the PSADT that will be assessed 18 to 20 months after initial patient accrual. Points estimate and exact confidence interval for the proportion of subjects who show 50% increase in PSADT will be reported.

Monitoring toxicity: Stopping rules for toxicity purpose are based on testing the null that toxicity level is less than 15% versus the alternative that it is actually greater than 35%. In this setting, we will carry two different but dependent looks. If 3 out of 10 show grade 3 toxicity or higher, the trial will stop (this will correspond to testing the toxicity hypotheses at level alpha=0.1 with a power of 68%); otherwise, another test for toxicity after we have a total of 17 subjects will be considered. If 5 out of 17 show a grade 3 toxicity or higher, the trial will stop (this will be equivalent to testing the toxicity hypotheses at level alpha =0.1 with a power 77%); otherwise we will proceed to full registration. The overall type I error for testing toxicity level is around 0.19.

Randomization to clinical arm: one-to-one.

Simon, Richard. "Optimal Two-Stage Designs for Phase II Clinical Trials," Controlled Clinical Trials, 1989, Volume 10, pages 1-10.

Addendum
PHASE II STUDY OF ADENOVIRUS/PSA VACCINE IN MEN WITH RECURRENT PROSTATE CANCER AFTER LOCAL THERAPY
Food and Drug Administration (FDA) Investigational New Drug (IND) #9706
Department of Defense, Prostate Cancer Research Program #A-14059.1

The following are reporting requirements and responsibilities of the Principal Investigator to the United States Army Medical Research and Materiel Command's (USAMRMC) Office of Research Protections (ORP), Human Research Protection Office (HRPO).

1. The protocol will be conducted in accordance with the protocol submitted to and approved by the USAMRMC ORP HRPO and will not be initiated until written notification of approval of the research project is issued by the USAMRMC ORP HRPO.

2. Accurate and complete study records will be maintained and made available to representatives of the U.S. Army Medical Research and Materiel Command as a part of their responsibility to protect human subjects in research. Research records will be stored in a confidential manner so as to protect the confidentiality of subject information.

3. All unanticipated problems involving risk to subjects or others, serious adverse events related to participation in the study and subject deaths related to participation in the study should be promptly reported by phone (301-619-2165), by email (hsrrb@det.amedd.army.mil), or by facsimile (301-619-7803) to the USAMRMC, Office of Research Protections, Human Research Protection Office. A complete written report will follow the initial notification. In addition to the methods above, the complete report will be sent to the U.S. Army Medical Research and Materiel Command, ATTN: MCMR-ZB-PH, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

4. Any deviation to the protocol that may have an effect on the safety or rights of the subject or the integrity of the study must be reported to the USAMRMC ORP HRPO as soon as the deviation is identified.

5. Major modifications to the research protocol and any modifications that could potentially increase risk to subjects must be submitted to the USAMRMC ORP HRPO for approval prior to implementation. All other amendments will be submitted with the continuing review report to the USAMRMC ORP HRPO for acceptance.

6. A copy of the approved continuing review report and the local IRB approval notification will be submitted to the USAMRMC ORP HRPO as soon as these documents become available. A copy of the approved final study report and local IRB approval notification will be submitted to the USAMRMC ORP HRPO as soon as these documents become available.

7. The knowledge of any pending compliance inspection/visit by the FDA, OHRP, or other government agency concerning clinical investigation or research, the issuance of Inspection Reports, FDA Form 483, warning letters or actions taken by any Regulatory Agencies including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements will be reported immediately to USAMRMC ORP HRPO. "

Roles and Responsibilities of Study Personnel:

David M. Lubaroff, PhD, Principal Investigator – Dr. Lubaroff, along with Dr. Williams, will manage all aspects of the trial, from assisting in patient recruitment, co-chairing the clinical trial meetings, to supervising the immunologic testing of the patients' sera and lymphocytes for anti-PSA immune responses. He will work together with Dr. Williams the Co-Principal Investigator on all important clinical issues for the trial.

Richard D. Williams, MD; Co-Principal Investigator – Dr. Williams will manage all patient care activities associated with this proposal and will work together with Dr. Lubaroff on managing the trial. Dr. Williams will function as co-chair of the weekly trial team meetings. He will be a major participant in the clinical management of prostate cancer patients that includes recruitment, patient management, assessing clinical response and any adverse events and long-term follow-up during the trial.

Fadi Joudi, MD, Co-Investigator – Dr. Joudi, as the second of two clinical urologists on the clinical trial team, will also participate in the recruitment and clinical management of the patients. As Chief of Urology at the Iowa City Veterans Affairs Medical Center (ICVAMC) Dr. Joudi will be an active participant in the treatment and follow-up of patients at that hospital. He will work closely with Dr. Vaena in that capacity.

Daniel Vaena, MD, Co-Investigator – Dr. Vaena is a Medical Oncologist who cares for prostate cancer patients following recurrences of their disease. He will assist in the recruitment and care of patients in the trial. As an attending oncologist at the Iowa City Veterans Affairs Medical Center (ICVAMC) Dr. Vaena will be an active participant in the treatment and follow-up of patients at that hospital. He will work closely with Dr. Joudi in that capacity.

Mark C. Smith, MD, Co-Investigator – Dr. Smith is a Radiation Oncologist who is responsible for radiation therapy of men with prostate cancer. He will assist in the recruitment and care of patients in the trial.

Tammy Madsen, BA, MPAS – **Study Coordinator** - Ms. Madsen, a Physician's Assistant in the Department of Urology, will be the study coordinator for the trial, and will coordinate activities with all of the clinical trial team, administer the informed consent document and obtain patient written consent, coordinate participant accrual, administer the vaccine to patients, and participate in the follow-up examinations.

Carlene Etscheidt, BSN, MSN – Clinical Trial Coordinator – Ms. Etscheidt participates in clinical trials in the Holden Comprehensive Cancer Center at the University of Iowa. She has and will continue to guide the clinical protocol through the institutional and federal other regulatory approval processes. She will also work closely with Ms. Madsen in follow-up visits of the patients.

Pamela Zehr, BSN, MSN - Clinical Trial Coordinator – Ms. Zehr also participates in clinical trials in the Holden Comprehensive Cancer Center at the University of Iowa. She will act as a backup for Ms. Etscheidt and also work closely with Ms. Madsen in patient follow-up.
Sara Miller, RN - Study Coordinator VAMC – Ms. Miller will be responsible for trial for patients enrolled in the trial at the VA Medical Center. She will work closely with all members of the trial team and attend the weekly meetings to discuss eligibility and follow-up of patients.

Gideon Zamba, PhD – Biostatistician – Dr. Zamba participated in the discussions pertinent to the development of the clinical protocols, performed the power analysis, and constructed the section on statistical considerations for the trial. He will assist in the data analysis and statistical interpretation of the patient data.

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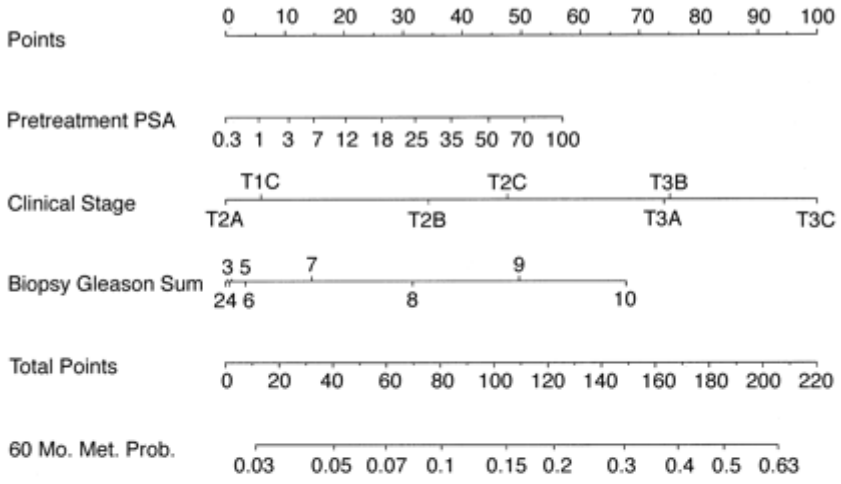
Katan Nomogram



Instructions for Physician: Locate the patient's PSA on the PSA axis. Draw a line straight upwards to the Points axis to determine how many points towards recurrence the patient receives for his PSA. Repeat this process for the other axes, each time drawing straight upward to the Points axis. Sum the points achieved for each predictor and locate this sum on the Total Points axis. Draw a line straight down to find the patient's probability of metastasis within 60 months.

Instruction to Patient: "Mr. X, if we had 100 men exactly like you, we would expect < predicted percentage from nomogram > to develop metastasis within 5 years following conformal radiation therapy. This prediction assumes you may need hormonal therapy, and that you might die of another cause first."

From: Katan, MW & Scardino, PT. Prediction of Progression: Nomograms of Clinical Utility. Clinical Prostate Cancer, 1: 90-96, 2002

[i] The term “life-threatening” in the definition of “serious” refers to an event in which the patient is at risk of death at the time of the event; it does not refer to an event, which hypothetically might have caused death if it were more severe.

[ii] Medically important conditions that may not result in death, be life-threatening or require hospitalization may be considered as SAE when, based upon appropriate medical judgment, they may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definition above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse. N.B.: The term “severe” is often used to describe the intensity (severity) of an event (such as: mild, moderate, or severe e.g., pain). The event itself may be of relatively minor medical significance (such as severe headache). This is not the same as “serious”, which is based on patient/event outcome or action criteria usually associated with events that pose a threat to patient’s life or vital functions. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

PHASE II STUDY OF ADENOVIRUS/PSA VACCINE IN MEN WITH HORMONE - REFRACTORY PROSTATE CANCER

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INDEX

1. INTRODUCTION

1.1 Background- Immunotherapy in Prostate Cancer

Prostate cancer is the second leading cause of cancer death among males in the United States. There will be an estimated 234,460 new diagnoses of prostate cancer made in the United States in 2006 (1). Treatments for organ-confined prostate cancer include radical prostatectomy and radiation therapy. When the cancer presents de novo, or recurs outside the prostate, first-line systemic treatments typically include hormonal blockade (with LHRH agonists or bilateral orchiectomy), which suppress testosterone levels, limit the growth of androgen-dependent cancer cells, and result in clinical tumor control. After a median time of 2 years, patients progress into a clinical hormone-refractory state, when the prostate specific antigen (PSA) levels rise despite castration, there is proliferation of androgen-independent cancer cells, and there is continued clinical tumor growth that becomes fatal. Therapeutic measures in this situation include further hormonal manipulations or the use of systemic chemotherapy, which has recently shown a small survival benefit in phase III trials. Approximately 30,000 Americans die from prostate cancer each year.

Immunotherapeutic approaches against prostate cancer have been investigated for several years. Most of these studies have concentrated on active non-specific therapy and adoptive or passive therapy, with only recent focus on the induction of antigen-specific immune responses. Viral vectors have been used successfully in both gene transfer and vaccine therapy studies (2). Replication-competent and replication-deficient adenoviruses expressing foreign proteins have been used to elicit immune responses to a variety of tumor antigens (3-7).

We have demonstrated that immunizations with adenovirus, carrying the human PSA gene, can induce vigorous anti-PSA T-cell responses and cause the destruction of PSA-secreting tumors in a pre-clinical mouse model of prostate cancer (8,9). Such active immunization against prostate-cancer associated antigens might be more effective than active non-specific or adoptive/passive immunotherapy. Therefore, we have pursued a vaccination strategy based on an adenovirus that carries the gene for prostate specific antigen (PSA). Results from our Phase I trial of adenovirus/PSA (Ad/PSA) vaccine (section 1.4, below) demonstrated that a single immunization of men with metastatic prostate cancer was able to induce anti-PSA T cell responses. The trial design was a dose escalation study with the vaccine administered subcutaneously (sc) either in an aqueous solution or in a collagen matrix (Gelfoam®). We now propose a Phase II clinical trial using the Ad/PSA vaccine, administered in multiple injections to prostate cancer patients with minimal disease burden.

1.2 Adenovirus vectors

Recombinant adenoviral vectors transduce a wide range of dividing and nondividing cells types, making this gene delivery system valuable as a tool for studying diseases, for vaccine therapy, and for potential clinical use (10). Recombinant adenovirus can be prepared and purified in high titers. In addition, wild-type adenovirus infections are extremely common in the general population, giving adenovirus a well-documented safety record (11). Moreover, adenoviruses are structurally stable and no adverse effects have been reported following the vaccination of US military recruits with wild types, demonstrating their safety for human use (11). Adenoviral vectors for gene therapy and vaccine therapy are adenoviruses which have been genetically modified to allow insertion of foreign genes and to render the virus replication-defective. Current vectors have a deletion in the E1 region or in both the E1 and E2 regions.

Adenoviral gene transfer has been used in a variety of experimental conditions that include transfers to the liver (12), lung (13), central nervous system (14,15), and to cancer cells (16).

There is evidence that the introduction of foreign transgenes by adenovirus induces immune responses to the transgene product, which become ultimately responsible for the elimination of the virus (17,18). While this is disadvantageous for insertion of functional genes into host cells, it is advantageous in the use of viruses carrying foreign genes as immunogens. In the vaccine therapy of cancer, active immunization against a murine colon cancer, breast cancer, and melanoma antigens have been induced by adenoviral vaccines (19-26).

The Ad/PSA vaccine used our laboratory and in our Phase I clinical trial was produced by inserting the gene for the full length pre-pro form of human PSA into a replication deficient adenovirus serotype 5. Replication deficiency was induced by deletion of the E1a and E1b genes of the virus. Details of the vaccine can be found in section 9 of this protocol. Approval for the use of the vaccine in the Phase I trial was obtained from the FDA under IND #9706.

In pre-clinical studies, our group has demonstrated that the Ad/PSA vaccine was able to induce stronger anti-PSA immune responses than other viral PSA vaccines. These include vaccinia viruses, both replication competent and replication deficient, and to a canarypox vaccine (Table 1), The frequency of PSA-specific CD8+ cells T cells generated by the Ad/PSA vaccine was greater than were generated by any of the other vaccines tested.. In addition to the superior immunizing property of the Ad/PSA, the incorporation of Gelfoam, a collagen matrix (section 1.3), has been shown in pre-clinical studies to enhance the ability of the vaccine to induce strong anti-PSA immune responses (8). Lastly, immunization of mice with Ad/PSA in matrix can induce anti-PSA responses even in the presence of high titer anti-adenovirus antibodies (8). This latter finding is important in light of the fact that most humans have pre-existing levels of anti-adenovirus antibodies as a result of prior natural exposure to the virus.

Table 1
Effector Cell Frequency Analysis (ELISPOT)

Vaccine	Virus	Frequency of PSA-Specific CD8+ T Cells
Ad/PSA*	Replication deficient adenovirus	1/455
Prostvac	Replication competent vaccinia	1/2028
NYVAC/PSA	Replication deficient vaccinia	1/3597
ALVAC/PSA	Canarypox	1/35,714

1.3 Gelfoam® Matrix

Gelfoam (Pharmacia & Upjohn Company, Kalamazoo, MI) is a medical device intended for application to bleeding surfaces as a hemostatic agent. It is a water-insoluble, off-white, non-elastic, porous, pliable product prepared from purified pork skin. The Gelfoam gelatin preparation is available either as a cross-linked sponge or as non-cross linked beads. It is able to absorb and hold within its interstices approximately 45 times its weight of blood and other fluids (26). The absorptive capacity of Gelfoam is a function of its physical size, increasing with increasing gelatin volume (27).

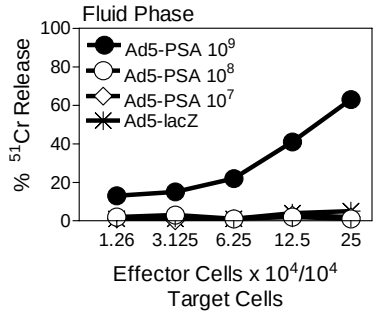
The mechanism of action of surface-mediated hemostatic devices is supportive and mechanical (27). Surface-acting devices, when applied directly to bleeding surfaces, arrest bleeding by the formation of an artificial clot and by producing a mechanical matrix that facilitates clotting (28). Jenkins et al have theorized that the clotting effect of Gelfoam may be due to release of thromboplastin from platelets, occurring when platelets entering the Gelfoam become damaged by contact with its myriad of interstices (29). Thromboplastin interacts with prothrombin and calcium to produce thrombin, and this sequence of events initiates the clotting reaction. The authors suggest that the physiologic formation of thrombin in Gelfoam is sufficient to produce formation of a clot, by its action on the fibrinogen in blood (29). The spongy physical properties of Gelfoam hasten clot formation and provide structural support for the forming clot (28,30).

Gelfoam has been used experimentally for the delivery of soluble proteins and drugs, including insulin, antibiotics, and growth factors (31-33). Gelfoam was used for sustained release of insulin in an ocular implant device (31). Delivery of insulin in solution had no effect on blood glucose levels. In contrast, the use of Gelfoam as a sustained release delivery agent provided measurable insulin activity for up to 10 hours after implantation. Glucose levels in the blood stabilized at 60% of the original value, whereas administration of insulin in eye drops had no effect.

MacDonald and Mathews (34) studied Gelfoam implants in canine kidneys and reported that it assisted in healing, with no marked inflammatory or foreign-body reactions. Jenkins and Janda (35) studied the use of Gelfoam in canine liver resections and noted that Gelfoam appeared to offer a protective cover and provide structural support for the reparative process. Correll et al (36) studied the histology of Gelfoam when implanted in rat muscle and reported no significant tissue reaction.

Gelfoam has been used as a hemostatic agent in dog prostate (37). In these studies no gross histological evidence of tissue damage or calcification was induced. In addition, these investigators demonstrated that placement of Gelfoam into the lumen of the bladder resulted in liquefaction of the Gelfoam without any evidence of calculogenesis. Finally, Bischoff and Goerttler (38) used Gelfoam in human prostate therapeutic embolization with success.

Our laboratory, in collaboration with Dr. Timothy Ratliff, has demonstrated that administration of the Ad/PSA vaccine in Gelfoam induces a stronger anti-PSA immune response (Figure 1). In our pre-clinical studies, immunization with the vaccine in an aqueous suspension induces strong immunity with 10^9 pfu with weaker immunity induced with 10^8 and 10^7 pfu. Use of Gelfoam permits the induction of strong responses at the lower dose of 10^8 pfu. In addition, strong anti-PSA T cell responses could be induced by immunization with the Ad/PSA vaccine in Gelfoam even in mice pre-immunized to adenovirus (Figure 2). In the Phase I clinical trial (section 1.4), the addition of Gelfoam to the vaccine immunization did not result in excess serious adverse events.



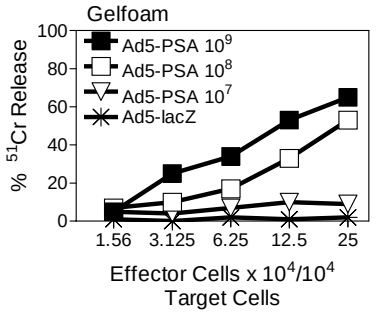


Figure 1 – Anti-PSA cytotoxic activity of spleen cells obtained from mice immunized with Ad/PSA in aqueous (fluid phase) versus Gelfoam.

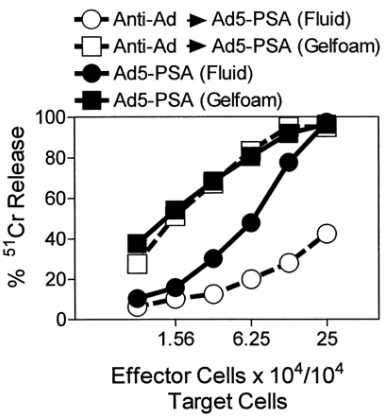


Figure 2. Effect of the presence of anti-adenovirus Ab on Ad5-PSA immunization with and without Gelfoam. Mice were injected i.p. with 10^9 PFU of Ad5-lacZ or PBS 2 wk before immunization with 10^9 PFU Ad5-PSA delivered s.c. in the fluid phase (PBS) or Gelfoam. CTL were tested against RM-11psa and RM-11neo to demonstrate PSA specificity. Cytolytic activity of each group against RM-11neo targets was <10% for all E:T ratios.

1.4 Phase I study

A Phase I clinical trial of the Ad/PSA vaccine has been completed in men with measurable metastatic prostate cancer, with the primary objectives of determining the toxicity profile and maximal tolerated dose (MTD). The ability of the vaccine to induce anti-PSA immune responses and any clinical responses was also evaluated. Funding for the Phase I trial was provided by multiple sources that include the Holden Comprehensive Cancer Center, the Department of Urology, and the Carver College of Medicine at the University of Iowa.

Eligible patients consisted of men with prostate cancer that had measurable metastatic disease, 90% of whom were stage D3. Prior therapies had included androgen depletion, ketaconazole, prednisone, Casodex, Taxotere, and external beam radiation, but the initiation of vaccine therapy was equal to, or greater than, 30 days after the most recent therapy. Patients were treated in successive dose levels and aqueous vs. matrix cohorts, according to the protocol plan. We were able to administer the maximum permitted dose of 10^8 pfu without any serious adverse events by vaccination of the first of 18 patients. These initial 18 patients were followed throughout the one-year period after injection. An additional 14 patients were treated at the MTD dose level, as planned in the protocol and confirmed by a letter to the FDA. The purpose of the additional patients was to have sufficient numbers of patients in the groups to statistically evaluate the anti-PSA immune responses induced by the Ad/PSA vaccine. In summary, 32 patients were treated in the study followed through the one-year period. Two additional patients were enrolled in the study (total number of enrolled patients, 34) but never received the vaccine and were therefore not evaluable. One patient chose to have radiation therapy instead of participating in the trial and the second was diagnosed with a second malignancy (melanoma) shortly after his enrollment in the phase I study.

1.4.1 **Phase I study results**

The median age of the patients was 70.2 years (range, 52 to 89). The vaccine was administered as an aqueous suspension or in a collagen (Gelfoam) matrix to 32 patients. Sixteen (16/32) or 50% of the patients exhibited grade 1 vaccine-related adverse events (AE), 1/32 (3.1%) that exhibited a grade 2 AE, and one patient exhibited a grade 3 AE which was a decrease in neutrophil count. There were no vaccine-related grades 4 or 5 AEs. The following Table 2 is a listing of all AEs, sorted by relationship to the vaccine injection.

Table 2
Ad/PSA Phase I Trial
Adverse Events – by Vaccine Relationship

Patient	System	Day of onset*	Event	Vaccine-related
AP005	Neural	21	Agitation	No
AP006	Musculoskeletal	13	Left hip and thigh pain	No
AP007	Musculoskeletal	22	Left back pain	No
AP007	Neural	14	Situational depression	No
AP009	GI	21	Constipation	No
AP009	Musculoskeletal	14	Joint aches, secondary to fall	No
AP009	Neural	11	Fall, causing head lacerations & loss of consciousness	No
AP010	GU	21	Proteinuria	No
AP010	Skin	21	Edema	No
AP014	Constitutional	22	Fatigue	No
AP014	Musculoskeletal	14	Leg pain (bone – femur)	No
AP015	Respiratory	1	Cough	No
AP016	GU	172	Left nephrolithiasis	No
AP016	GU	183	New primary tumor – papillary bladder	No
AP016	Immune	8	Flu-like symptoms	No
AP016	Musculoskeletal	4	Bone pain – left leg	No
AP016	Musculoskeletal	21	Decreased left leg strength	No
AP018	Musculoskeletal	8	Bilateral rib pain	No
AP018	Neural	8	Insomnia	No
AP020	Neural	168	cord compression	No
AP022	GI	2	constipation	No
AP022	Hematologic	14	decreased lymphocyte count	No
AP022	Hematologic	63	decreased WBC & neutrophils	No
AP022	Musculoskeletal	5	increased bone pain	No
AP022	Neural	16	depression	No
AP023	Musculoskeletal	20	pain in left buttocks	No
AP025	Musculoskeletal	16	bone pain	No
AP026	Cardiovascular	14	Lower extremity edema	No
AP027	GI	1	GI cramping	No
AP027	GI	1	constipation	No
AP034	Respiratory	prior	Edema, wheezing (change in inhaler in same time period)	No
AP035	Constitutional	64	Weight loss	No
AP035	GI	50	Constipation	No
AP035	GU	22	Proteinuria	No
AP035	Hematologic	15	Lymphopenia	No
AP035	Musculoskeletal	22	Increased alkaline phosphatase	No
AP037	GI	73	Nausea	No
AP018	Skin	1	Injection site tenderness	Possible
AP019	Cardiovascular	1	Hypotension	Possible
AP019	Constitutional	1	fever	Possible
AP019	Constitutional	14	fatigue	Possible
AP019	GU	1	Proteinuria	Possible
AP020	GI	21	increased alkaline phosphatase	Possible
AP020	GU	21	ketonuria	Possible

AP020	Metabolic	14	hyponatremia	Possible
AP020	Metabolic	14	hyperglycemia	Possible
AP020	Respiratory	9	viral symptoms	Possible
AP005	Musculoskeletal	1	Left inguinal pain	Possibly
AP008	GU	21	Proteinuria	Possibly
AP008	Respiratory	3	Cold symptoms	Possibly
AP010	Hematologic	1	Decrease in absolute neutrophils count	Possibly
AP027	Liver	21	increased AST	Possibly
AP036	Hematologic	1	Anemia	Possibly
AP036	Hematologic	63	Anemia	Possibly
AP037	Hematologic	1	Lymphopenia	Possibly
AP002	Musculoskeletal	3	Groin pain	Unlikely
AP002	Neural	3	headache	Unlikely
AP002	Skin	1	Itching	Unlikely
AP003	Pulmonary	21	Pleural effusion	Unlikely
AP006	Constitutional	14	Chills	Unlikely
AP006	Neural	14	Migraine headache	Unlikely
AP012	Cardiovascular	21	Hypotension	Unlikely
AP012	GI	0	Heartburn	Unlikely
AP012	Neural	21	Dizziness	Unlikely
AP014	Hematologic	82	Anemia	Unlikely
AP015	Cardiovascular	137	Myocardial infarction	Unlikely
AP018	Respiratory	43	DOE?	Unlikely
AP021	Hematologic	1	decreased lymphocytes	Unlikely
AP021	Hematologic	63	thrombocytopenia	Unlikely
AP023	Immune	16	urinary tract infection	Unlikely
AP023	Liver	21	elevated AST	Unlikely
AP024	Hematologic	14	anemia	Unlikely
AP024	Hematologic	1	anemia	Unlikely
AP025	GI	85	abdominal distention, constipation, apparently related to metastatic disease in the periaortic lymph nodes and periprostatic tumor mass	Unlikely
AP025	Hematologic	14	thrombocytopenia	Unlikely
AP025	Neural	122	Headache	Unlikely
AP027	Immune	21	Infection	Unlikely
AP034	Cardiovascular	14	Hypotension/dizziness	Unlikely
AP034	Musculoskeletal	14	Back pain	Unlikely
AP036	Cardiovascular	63	Edema – bilateral ankles	Unlikely
AP002	Skin	0	hematoma at injection site	Yes
AP003	Skin	0	Bruising at injection site	Yes
AP004	Skin	0	Ecchymosis at injection site	Yes
AP005	Skin	0	Ecchymosis and erythema at injection site	Yes
AP008	Skin	0	Ecchymosis at injection site	Yes
AP009	Skin	0	Ecchymosis at injection site	Yes
AP018	Skin	0	Ecchymosis	Yes
AP025	Skin	0	Ecchymosis at injection site	Yes
AP026	Skin	0	Erythema at injection site	Yes
AP034	Skin	0	Pain at injection site	Yes

* day 0 = day of injection; day 1 = day after injection

We measured the anti-PSA immune responses, both antibody and T cell, in all patients enrolled in the study. Antibody responses to PSA were measured by the binding to PSA-secreting cell lines using the method adapted from Cavacini, et al. (39). Results of those analyses demonstrated that 57% of men immunized with the Ad/PSA vaccine developed measurable anti-PSA antibodies. ELISPOT assays were utilized to measure anti-PSA T cell responses. The results, depicted in Table 3, demonstrate that of the 32 patients, 18 (56.3%) developed anti-PSA T cell responses. The addition of Gelfoam did not appear to affect the development of anti-PSA responses, but in this Phase I study the numbers of patients in each group was too small to make statements of statistical significance of the data. These results demonstrate the ability of men with late stage metastatic prostate cancer, injected one time with Ad/PSA, to respond to the vaccine with the production of anti-PSA T cells.

Table 3
ELISPOT Analysis of Anti-PSA T Cell Immune Responses

Patient Number	Dose/ Vehicle	Response	Frequency	
			Pre-Immunization	**Post- Immunization**
AP-002	10^6-aqueous	-	1/985,000	1/258,571
AP-004	10^6-aqueous	-	0	1/311,111
AP-007	10^6-aqueous	+	1/46,901	1/14,804
AP-003	10^6-matrix	+	1/90,000	1/8075
AP-005	10^6-matrix	+	$1/1.2x10^6$	1/152,353
AP-006	10^6-matrix	+	1/93,103	1/15,762
AP-008	10^7-aqueous	-	1/100,000	1/97,419
AP-010	10^7-aqueous	-	0	0
AP-013	10^7-aqueous	+	0	1/60,000
AP-009	10^7-matrix	+	1/52,535	1/30,566
AP-012	10^7-matrix	-	1/13,488	1/254,286
AP-014	10^7-matrix	+	1/562,500	1/120,000
AP-015	10^8-aqueous	+	$1/1.4x10^6$	1/780
AP-016	10^8-aqueous	+	0	1/130,000
AP-017	10^8-aqueous	+	0	1/124,375
AP-025	10^8-aqueous	-	0	0
AP-026	10^8-aqueous	-	0	1/560,000
AP-027	10^8-aqueous	+	0	1/26,364
AP-029	10^8-aqueous	+	0	1/333
AP-032	10^8-aqueous	+	1/250,000	1/3793
AP-018	10^8-matrix	-	0	0
AP-019	10^8-matrix	+	1/14,235	1/336
AP-020	10^8-matrix	+	1/8824	1/1802
AP-021	10^8-matrix	+	1/689	1/431
AP-022	10^8-matrix	+	0	1/180,000
AP-023	10^8-matrix	-	1/320,000	0
AP-030	10^8-matrix	+	1/666.667	1/32,000
AP-034	10^8-matrix	+	1/80,000	1/15,152
AP-035	10^8-matrix	+	1/5295	1/2459
AP-036	10^8-matrix	+	1/62,000	1/9487
AP-037	10^8 matrix	-	$1/2.1x10^6$	1/965,000

The effects of vaccination on serum PSA and on patient survival were also evaluated as a secondary endpoint in the phase I trial. Although there was no sustained decline in individual PSA levels, the PSA doubling times (PSADT, calculated based on 3 pre-enrollment consecutive

PSA measurements) were reduced in 54 percent of the patients compared to pre-vaccine administration, with the best responses occurring in patients immunized with the highest dose of the vaccine (Table 4)**.** In addition, published survival nomograms for patients with hormone refractory prostate cancer were applied to patients in this phase I trial (40,41). Table 5 shows that 57% of all patients at all doses, whether injected with the vaccine as an aqueous suspension or in the collagen matrix, had a survival time longer than that predicted by the nomogram, The range of increased survivals in the different groups was 33% to 100%.

Table 4
PSA Doubling Time (DT) in Phase I Patients

Vaccine Dose & Vehicle	Percent iNcreased PSA DT
10^6 aqueous	33%
10^6 matrix	33%
10^7 aqueous	67%
10^7 matrix	67%
10^8 aqueous	25%
10^8 matrix	62%
Total	**54%**

Table 5
Ad/PSA Phase I Trial
Predicted and Actual Patient Survival

Vaccine Dose	Vehicle	Percent with Longer Than Predicted Survival
10^6	Aqueous	33%
10^6	Matrix	67%
10^7	Aqueous	100%
10^7	Matrix	33%
10^8	Aqueous	56%
10^8	Matrix	50%
Overall		**57%**

1.5 Other immunotherapy clinical trials for prostate cancer

The last several years have seen an increase in the number of clinical trials using vaccine immunotherapy for the treatment of prostate cancer. The trials have used a variety of target antigens that have been shown to be associated with prostate and prostate cancer cells. These include PSA (39,42-49), prostatic acid phosphatase (PAP) (50-53), prostate specific membrane antigen (PSMA) (54-56), telomerase (hTERT) (57,58), Thomsen-Friedenreich antigens (59), mucins (60), carbohydrates (61), and HLA-associated peptides (62). A variety of vectors have been used in the immunization process that include dendritic cells (45,50-58,63), vaccinia virus (39,42,43,47,49), fowlpox virus (39,47), liposomes (44), plasmids, (48), and chemical conjugates (59-61).

Recently, Kaufman et al. recently reported the results of a phase II trial (ECOG 7897) with a prime/boost vaccine using vaccinia virus and fowlpox virus expressing human PSA in patients with hormone-dependent prostate cancer (64). Sixty-four eligible patients with biochemical progression after local therapy were randomly assigned to three treatment arms: (A) fowlpox-PSA (rF-PSA) by intramuscular injection every six weeks for four doses, (B) rF-PSA for three

doses followed by vaccinia-PSA (rV-PSA) given by intradermal injection, or (C) rV-PSA followed by three rF-PSA vaccines. Dreicer et al. reported a randomized phase II study with a recombinant Modified Vaccinia Ankara virus which expresses both MUC1 and IL2 (TG4010) (65). MUC1 is a glycoprotein associated with several malignancies. Eligible patients were required to have no evidence of metastatic disease following curative intent local therapy, evidence of PSA failure (PSA over 2 ng/ml) and PSA doubling time (PSA-DT) less than 10 months. Arm 1 had TG4010 injected sc weekly, at a dose of 10^8 pfu, for six weeks, then every three weeks. Arm 2 had 10^8 pfu TG4010 injected sc every three weeks. Therapy was continued to disease progression or to a maximum of 36 weeks. Lastly, Small et al. recently presented the results of a phase III trial with APC8015, an immunotherapy cellular product consisting of autologous peripheral blood mononuclear cells enriched for a dendritic cell fraction pulsed with PA2024, a Prostatic Acid Phosphatase (PAP)-GM-CSF construct (66). Patients with asymptomatic, metastatic hormone-refractory prostate cancer were randomized (2:1) to receive APC8015 (n=82) or placebo (n=45) every 2 weeks x 3.

ECOG is currently planning a phase III trial using the Vaccinia virus (PROSTVAC-V/TRICOM) followed by Folwlpox virus vaccination (PROSTVAC-F/TRICOM) with GM-CSF, compared with placebo vaccine plus GM-CSF in patients with hormone-refractory prostate cancer with absence of metastatic disease (ECOG 1805, PARADIGM).

In summary, the results from these trials vary in terms of patient populations studied (hormone dependent vs. independent) and in levels of positive results, which include the induction of antigen-specific immune responses, decreases in levels of serum PSA and in rates of change in PSA velocity, and measures of clinical responses. Thus far no single vaccine immunotherapy has proven to be definitely superior to others in terms of clinical benefit, and other phase II and III trials continue to be planned or conducted. The results of some of these vaccine trials raise the question that an increase in PSADT may in the future represent a possible surrogate marker for increased time to progression, or overall survival in immunotherapy studies, and that absolute PSA responses may not constitute an obligatory step for the ultimate demonstration of clinical benefit of immunotherapy approaches in prostate cancer. Furthermore, the T-cell stimulation index may have important correlation with clinical vaccine efficacy, as seen in the phase III trial by Small et al.(66). These developing notions further support the current proposal for clinical development of our Ad/PSA vaccine, also based on the results of our prior phase I trial.

1.6 Proposed phase II clinical trial: rationale

Based on the significant pre-clinical activity of the Ad/PSA vaccine in generating tumor-specific T cells, and the encouraging safety and efficacy results from our phase I study, we propose to continue the clinical development of the Ad/PSA vaccine with the performance of the current phase II trial. It is our contention that the vaccine product and the method of immunization set this therapy apart from other ongoing prostate cancer investigational immunotherapeutic approaches. Specifically, the incorporation of Gelfoam, not present in other vaccine preparations, enhances the induction of strong anti-PSA responses. Immunization of mice with Ad/PSA in Gelfoam matrix was able to induce anti-PSA responses even in the presence of high-titer anti-adenovirus antibodies. Notably, most humans naturally possess high titers of anti-adenovirus antibodies due to natural exposure to adenoviruses.

We plan to enroll prostate cancer patients with hormone-refractory metastatic disease into this Phase II clinical trial. This is similar to the population that constituted the majority of patients in our Phase I toxicity trial of the Ad/PSA vaccine. Patients in this trial will have low

burden of disease, despite the fact that they are hormone refractory, i.e., have negative bone scans and/or low serum PSA. We will be comparing the clinical and immunologic response of these patients with two other patient populations in the trial. The latter populations, described in a separate protocol, will be patients with newly recurrent disease, either hormone naïve or during androgen depletion therapy. Although the patients with recent recurrences will have a smaller tumor burden than will the patients in this protocol that have metastatic disease, the observation period to detect a therapeutic effect of the vaccine will be shorter in the latter patient population than the former population. It is for this important reason that we will enroll patients with metastatic disease on this protocol. Patients will be eligible if the have recent evidence of hormone refractory disease (D3) and either (a) have a positive bone scan with a PSA doubling time of $\geq$12 months, a total PSA of <5 ng/ml, and asymptomatic; or (b) have a negative bone scan with any PSA doubling time, asymptomatic, and not a candidate for chemotherapy.

We will enroll and treat patients at two affiliated and adjacent medical centers, the University of Iowa Hospitals and Clinics (UIHC) and the Iowa City Veterans Affairs Medical Center (ICVAMC). There is a single Institutional Review Board (IRB) that approves protocols for both institutions. After the University of Iowa's IRB has approved the protocols the Research and Development Committee of the ICVAMC meets to provide their approval for the study.

Patient Recruitment: The UIHC and ICVAMC draw from a large catchment area that will insure the enrollment of sufficient numbers of men into the protocol. We will use recruitment methods similar to the methods used to obtain subjects for our Phase I clinical trial. That includes contacting patients that are being or have been treated in the Departments of Urology, Internal Medicine, and Radiation Oncology at the UIHC and ICVAMC and letters written to urologists, oncologists, and medical oncologists in Iowa and the neighboring states of Wisconsin, Illinois, Missouri, Nebraska, Minnesota, and South Dakota. Drs. Williams and Joudi will assist in the recruitment of patients from the Department of Urology, Dr. Vaena from Internal Medicine, and Dr. Smith from Radiation Oncology.

A physician from outside the UIHC or ICVAMC may inform patients of the study. If a patient expresses interest in learning more about the trial, the physician will provide him with contact information for the clinical trial team. When a patient contacts the clinical trial team, a member of the trial team will briefly describe the study and request the patient's mailing address in order to mail him a copy of the study informed consent form.

After the patient has had time to become familiar with the study and discuss it with his family, a member of the research team will follow up with a telephone call to discuss the study and answer any questions the patient may have. At this time, if the patient is still interested in participating, he will be asked to provide verbal consent to allow the research team to review information from his medical record to evaluate his eligibility for the trial. The researcher will document the verbal consent process in the research file, and will contact the patient's physician to request the medical record for review. The patient will be required to sign a HIPAA privacy form and a Release of Medical Information form at his physician's office prior to the physician releasing his records to the research team.

After reviewing the relevant portions of the medical record, a member of the research team will again contact the patient. Eligible individuals will be scheduled for a visit to the UIHC or ICVAMC to undergo additional eligibility testing. At that visit, informed consent for participation in the clinical trial will be obtained. The subject will then be further evaluated for study eligibility through a medical history and physical exam, blood tests, and scans. Final determination of eligibility will occur at a weekly meeting of the clinical trial team.

2. OBJECTIVES

2.1 Primary Objective

To evaluate the response rates (PSA responses and changes in PSADT) following immunization with the Ad/PSA vaccine using a prime-boost immunization strategy, in patients with hormone refractory metastatic disease.

2.2 Secondary objectives

2.2.1 To evaluate the development of anti-PSA immune responses in study patients.

2.2.2 To evaluate biochemical (PSA recurrence) and radiographic (bone scans) time to progression and overall survival in evaluable patients receiving the Ad/PSA vaccine.

3. SELECTION OF PATIENTS

As described in Section 1.6 prostate cancer patients with hormone refractory metastatic disease will be enrolled in the study.

3.1 Inclusion criteria:

3.1.1 Men with prostate cancer who present with evidence of hormone refractory disease (D3).

3.1.2 Men with a positive bone scan, a PSA doubling time of $\geq$12 months, and a total PSA of <5 ng/ml, and asymptomatic.

3.1.3 Men with a negative bone scan with any PSA doubling time and asymptomatic.

3.1.4 Scans must be obtained within 6 weeks of entry into the trial.

3.1.5 Written informed consent.

3.1.6 Age $\geq$ 18 years.

3.1.7 Required laboratory values (obtained within 2 weeks of study entry)

3.1.7.1 Serum creatinine $\leq$ 2.0 mg/dL

3.1.7.3 Adequate hematologic function: granulocytes $\geq$ 1800 per mm^3, platelets $\geq$ 100,000 per mm^3, WBC $\geq$3700, and lymphocytes >590.

3.1.7.4 Adequate hepatocellular function: AST <3x normal and bilirubin <1.5 mg/dl.

3.1.7.5 Castrate levels of testosterone of <5 ng/ml.

3.2 Exclusion criteria:

3.2.1 Active or unresolved infection.

3.2.2 Parenteral antibiotics <7 days prior to study entry.

3.2.3 Evidence of prior or current CNS metastases. Specific imaging is not necessary in the absence of signs or symptoms.

3.2.4 Co-morbid medical conditions which would result in a life expectancy (participation) of less than 1 year.

3.2.5 Patients with compromised immune systems; congenital, acquired, or drug-induced (immunosuppressive agents) will be excluded from the study. Use of prednisone at doses higher than 10 mg daily (or equipotent steroid doses) for more than 7 days within the last 3 months is not allowed.

3.2.6 No-pre-existing malignancies that required treatment within the past 5 years except for basal or squamous cell cancers of the skin.

3.2.8 Prior participation in any vaccine studies for any disease.

3.2.9 Prior chemotherapy, defined as prior cytotoxic chemotherapy for prostate cancer (or any cancer unless more than 5 years have elapsed). Examples of cytotoxic chemotherapy are mitoxantrone/prednisone and taxanes. Drugs such as Casodex or ketoconazole treatment must have been completed at least 6 weeks prior to registration.

3.2.10 The inability to understand the language and the clinical protocol.

3.2.11 Allergy or religious objection to pork products; Gelfoam is produced from pork.

4. Registration Procedures

4.1 All patients will be registered through the Department of Urology at the University of Iowa Hospitals and Clinics (UIHC) or the Urology Service at the Iowa City Veterans Affairs Medical Center (ICVAMC).

4.2 Patients who are candidates for enrollment into the study will be evaluated for eligibility by the clinical investigators to ensure that the criteria outlined in Section 3 have been satisfied and that the patient is eligible for participation in this clinical investigation. The University of Iowa will provide a patient eligibility case report form for this evaluation.

4.3 Informed Consent - Signed informed consent for enrollment in this protocol will be obtained from eligible patients by the attending physician, study coordinator, or clinical trial coordinator before the start of research intervention. At the preadmission consultation, patients will be fully informed of the purpose and potential risks and benefits of participating in the study. Patients have the opportunity to have questions answered to their satisfaction before signing the consent.

4.4 Eligible patients must be registered Monday through Friday between 8:00 a.m. and 4:30 p.m. (Central Time) by calling Carlene Etscheidt, BSN, MSN or Pamela Zehr, BSN, MA the University of Iowa Clinical Cancer Center, Iowa City, Iowa, 319-356-1228 or 319-353-8914 respectively. Patients at the ICVAMC will be registered by calling Sara Miller, BSN at 319-338-0581, ext. 7519. Information from the eligibility form will be provided by the investigator or the investigator's research staff to the University of Iowa Cancer Center at this time, and the patient will be registered and assigned a unique patient number.

4.5 No patient may be enrolled or begin research intervention prior to registration and assignment of a patient number. As a follow-up, University of Iowa Cancer Center will provide the investigator with written confirmation of each patient's registration.

4.6 All investigators will be notified by the Chair of the Protocol Review and Monitoring Committee or by the trial's Data and Safety Monitoring Board if the study is placed on administrative hold, and when the study is completed or closed to further patient enrollment.

4.7 Patients must begin the vaccine protocol within 7 days of registration.

5. RESEARCH INTERVENTION PLAN

5.1 Administration Schedule

Ad/PSA

All patients will receive three injections of 0.125 ml. of the Ad/PSA subcutaneously in the right thigh. The dose of the vaccine, based upon our results from the Phase I trial, will be 1 x 10^8 pfu (4.4 x 10^9 particles) in the Gelfoam matrix. The Gelfoam comes in sterile patient-ready packages. The virus will be suspended in sterile saline and the Gelfoam powder added in a ratio of 30 mg of powder per ml. of virus suspension. Injections will be spaced apart by 30 days, such that each patient will receive the vaccine on days 0, 30, and 60. The use of the matrix has been shown in collaborative pre-clinical experiments to enhance infection of host cells by the virus. Results from the Phase I trial indicated that the injection of the vaccine in Gelfoam did not produce any adverse events greater than those produced by the vaccine in an aqueous suspension. The vaccine induced anti-PSA immune response in patients injected as an aqueous or Gelfoam vaccination. Injections will be carried out in the University of Iowa General Clinical Research Center (GCRC). Each subject will be housed in the CRC for 24 hours and observed for early signs of toxicities. Tests, indicated in the table on page 23, will be carried out to be certain that no serious side effects are induced by the vaccine.

5.2 Design and Stages

The ideal design would be a two-stage design of Simon (1989) requiring 32 patients. But due to the complexity of the trial, a more conservative approach consisting of using 32 patients in a one-stage design at efficacy level and a two looks at toxicity level as means of stopping rules will be carried. By using a one-stage design at efficacy endpoint, we are making the probability of early termination for efficacy purposes to be zero. The reason for this design is due to the nature of the research intervention as outlined in section 10. Stopping design based on toxicity is as follow: one look after 17 subjects are in the trial and another look after 25 subjects are enrolled. If 5 out of 17 show grade 3 toxicity or higher, the trial will stop. Otherwise, an additional 8 subjects will enter the trial. If 7 out of 25 show grade

3 toxicity or higher, the trial will stop otherwise we will proceed to full registration of 32 patients to assess efficacy.

6. Adverse Events

Toxicity will be graded according to the NCI common toxicity criteria "Common Terminology Criteria for Adverse Events" (NCI-CTCAE v3.0 can be accessed at website: http://ctep.cancer.gov/forms/CTCAE_Index.pdf). Non-hematologic dose limiting toxicity (DLT) will be defined as grade III non-hematologic toxicity. Hematologic DLT will be declared if patients develop grade IV or grade III and fail to recover their absolute neutrophil count (ANC) and platelets to Grade I/II levels after 5 weeks, unless such failure is due to progressive tumor.

6.1 Definitions

Adverse Event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this research intervention. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease* temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

This will also include intercurrent diseases and accidents observed during the research intervention period as well as corresponding events during drug-free, pre- and post-intervention periods, under placebo or in a reference group receiving drug or non-drug therapy.

Serious adverse event (SAE) is any untoward medical occurrence that:

a. results in death
b. is life-threatening[B]
c. requires inpatient hospitalization or prolongation of existing hospitalization
d. results in persistent or significant disability or incapacity
e. is a congenital anomaly / birth defect or
f. is another medically important condition.[C]

6.2 Procedures of documentation of AEs

[B] The term "life-threatening" in the definition of "serious" refers to an event in which the patient is at risk of death at the time of the event; it does not refer to an event, which hypothetically might have caused death if it were more severe.

[C] Medically important conditions that may not result in death, be life-threatening or require hospitalization may be considered as SAE when, based upon appropriate medical judgment, they may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definition above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse. N.B.: The term "severe" is often used to describe the intensity (severity) of an event (such as: mild, moderate, or severe e.g., pain). The event itself may be of relatively minor medical significance (such as severe headache). This is not the same as "serious", which is based on patient/event outcome or action criteria usually associated with events that pose a threat to patient's life or vital functions. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

All AEs occurring during the study must be documented, regardless of the assumption of a causal relationship, on the respective AE CRF. All events, which occurred after signed informed consent, should be documented. The investigator should ensure that all events are recorded that occurred within at least 4 weeks after the last exposure to the study drug.

Documentation of AEs includes: date of onset and offset, intensity, frequency, seriousness, related interventions and outcome. The investigator will also evaluate the probability of a causal relationship of the adverse event to the study medication as being: “definite, probable, possible, unlikely, or unrelated.”

All unanticipated problems involving risk to subjects or others, serious adverse events related to participation in the study and subject deaths related to participation in the study will be promptly reported by phone (301-619-2165), by email (hsrrb@det.amedd.army.mil), or by facsimile (301-619-7803) to the USAMRMC, Office of Research Protections, Human Research Protection Office. A complete written report will follow the initial notification. In addition to the methods above, the complete report will be sent to the U.S. Army Medical Research and Materiel Command, ATTN: MCMR-ZB-PH, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

The medical monitor is required to review all unanticipated problems involving risk to volunteers or others, serious adverse events and all volunteer deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the medical monitor should comment on the outcomes of the event or problem, and in the case of a serious adverse event or death, comment on the relationship to participation in the study. The medical monitor should also indicate whether he/she concurs with the details of the report provided by the study investigator. Reports for events determined by either the investigator or medical monitor to be possibly or definitely related to participation and reports of events resulting in death should be promptly forwarded to the HSRRB.

Expedited reporting

The investigator must immediately report serious adverse events (SAE) occurring or observed during the course of the study and within 4 weeks of last administration of the study drug to the FDA, IRB, OBA, and GCRC.

After notifying the proper agencies by telephone of an SAE within 24 hours of the knowledge of the event’s occurrence, the “Serious Adverse Event Report ”must also be sent by fax to the agencies whether or not complete information is available at the time. If complete information is unavailable the investigator must provide follow-up information to the agencies as soon as it is known.

In particular, the investigator must inform the agencies by phone and fax within 24 hours of occurrence of immediately life-threatening SAEs or SAEs with fatal outcome. SAEs must be reported to the site’s IRB according to the IRB’s requirements.

Important: The investigator must report any SAE to the FDA, IRB, OBA, and GCRC, regardless of causality.

Reports will be evaluated by the Medical Monitor/Sponsor. FDA/HPB and investigators will be informed as required by the regulations. The same information will also be made available to all participating investigators as well as to other investigators participating in different clinical trials utilizing the same study medication.

It should be noted that, although the Ad/PSA vaccine contains the gene for PSA, there is no need for patients to utilize contraceptive practices. The adenovirus will not be transmitted from patient to his partner.

7 MEASUREMENT OF CLINICAL AND IMMUNOLOGICAL EFFICACY

7.1 Methods of Malignant Disease Evaluation - Each patient will have a baseline evaluation prior to the injection of the Ad/PSA vaccine. The measurements will include temperature, weight, serum PSA, blood chemistries, a quantitative bone scan for bone metastases, chest x-ray, and CT for soft tissue metastases, and performance status for quality of life.

Patients will be seen in the GCRC (see Table 6 for schedule). The injection site will be examined for evidence of erythema, induration and necrosis and patients will have their temperature and weight recorded and interviewed to determine whether they experienced any adverse reactions. Blood samples will also be taken for measurement of PSA and anti-PSA antibodies (see Table 6). At the 6 month, 12 month, and subsequent semi-annual visits each patient will be evaluated using the measurements listed for the baseline visit.

Because changes to the clinical status of the patients will be prolonged, long-term follow up will be important. Therefore, patients will return to the clinic every six months following the 12 month post-vaccination visit. The visits will continue indefinitely unless the patient demonstrates signs of progressive disease.

7.2 Scans – Bone scans will be performed every three months for the first year and every 6 months thereafter. If there is measurable disease by CT scan at registration then CT scans will also be performed using the same schedule as the bone scans.

7.3 Use of Serum PSA for Disease Evaluation – Based upon our pre-clinical experiments and the results from the Phase I clinical trial we expect the immunized men to produce anti-PSA antibodies. The levels of antibody will be measured by a flow cytometry assay as described by Cavacini, et al. used in our Phase I clinical trial (40). We will also explore the use of a second serum marker for prostate cancer, hK2 in collaboration with Donald Tindall, Charles Young, and George Clee at the Mayo Clinic. Investigators at Mayo, along with Hybritech, Inc. have been exploring hK2 and published a number of papers in recent years on the subject (67-70). Patient sera from each clinic visit will be sent to Mayo where they will measure the levels of hK2. We will use the data to evaluate the effect of anti-PSA antibodies on both PSA and hK2 in the sera of vaccinated patients.

PSA measurements, CT and bone scans are routinely used to follow disease recurrence and/or progression in individual prostate cancer patients and as such would be considered standard of care. Laboratory measurements such as hematology, liver function and kidney function chemistries, while are routinely used to follow the health of a prostate cancer patient, would not normally be performed at the frequency proposed in this trial to assess possible vaccination toxicities. Therefore, they would be considered part of the research protocol.

7.4 Experimental Evaluation of the Ad/PSA Vaccination

7.4.1 Blood will be collected prior to, and at each visit after, the injection of the Ad/PSA vaccine. Two separate samples will be collected; one in red top tubes to allow collection of serum from coagulated blood and a second in heparinized tubes to permit collection of lymphocytes.

7.4.2 Levels of PSA, hK2, anti-PSA antibodies, and anti-adenovirus antibodies will be measured in the serum.

7.4.3 Anti-PSA T cell immune responses will be measured by ELISPOT analysis using the methods developed for, and used in, our Phase I clinical trial. In addition to measuring the anti-PSA T cell activity, we will also measure anti-adenovirus T cell activity as well as reactivity to stimulation with cytomegalovirus (CMV). A non-specific stimulus will be provided by PMA and ionomycin for each patient's lymphocytes.

7.5 Definitions of Response –

7.5.1 Primary Endpoint - PSA doubling-time response

7.5.1.1 Definition: a 50% increase in the PSADT compared to pre-enrollment PSADT.

7.5.1.2 PSADT will be calculated based on the MSKCC calculator, available at http://www.mskcc.org/mskcc/html/10088.cfm.

7.5.1.3 PSADT response will be measured at 9 and 18 months after initiation of the research intervention.

7.5.1.4 Three measurements of PSA, spaced at least 2 weeks apart, will be required prior to study enrollment. Post-intervention PSADT will be based on PSA levels at 3, 6 and 9 months (9 month PSADT calculation) and 3,6,9,12,15,18 month levels (18 month PSADT calculation).

7.5.2 Secondary Endpoint – PSA response

7.5.2.1 Definition: a 50% reduction in the pre-research intervention PSA value, verified with a second measurement 30 days later.

7.5.3 Progression:

7.5.3.1 In men with previously negative bone scan at entry - positive bone scan and a rising PSA. A minimum of three measurements are necessary to document a rising PSA.

7.5.3.2 In men with previously positive bone scan at entry - doubling of PSA and/or changes in bone scan. A minimum of three measurements are necessary to document the PSA doubling.

7.5.3.3 In men with measurable disease by CT at entry – progressive change

7.5.4 Onset of Response – The time between initiation of therapy and the onset of PR or CR.

7.5.5 Duration of Response – Time from onset of PR or CR, whichever occurs first, (even if the patient later has a CR) until objective evidence of progression.

7.6 Timing of Toxicity Assessments - Toxicity assessment will occur as stated in the calendar. We will wait for the last patient of stage I to reach the 90 day toxicity assessment date (and review of safety data) prior to proceeding with stage II as outlined in the statistical plan.

8 STUDY PARAMETERS

8.1 Scans or x-rays used to document measurable or evaluable disease should be done with 4 weeks prior to study entry

8.2 CBC with differential, LFT's should be done ≤2 weeks before study entry. Castrate levels of testosterone

8.3 All chemistries should be done ≤2 weeks before the study entry, unless specifically required on day 1 as per protocol. If abnormal, they must be repeated within 48 hours prior to study entry.

8.4 Hgb, Hct, WBC, Plt should be done ≤2 weeks before study entry but, if abnormal, they must be repeated <48 hours prior to study entry.

8.5 REMOVAL OF PATIENTS FROM STUDY (Criteria for discontinuation of a patient's study participation)

8.5.1 Adverse events: In the event of a vaccine-associated unmanageable or irreversible toxicity, that would include hematological and non-hematological toxicities, the investigator will withdraw a patient from further research intervention and notify the Study Chair immediately. *In addition, the FDA and the IRB will be notified of the adverse events.* If unmanageable or irreversible toxicities occur the patients will receive the best possible medical care according to the recommendations of the treating physicians. The treatment plan will depend upon what unmanageable or irreversible toxicities may occur. In the event of an emergency the patient will be instructed to seek immediate care by calling 911, his local physician, the University of Iowa or Iowa City VA Medical Center urology staff on call.

8.5.1.1 Management of Toxicities

8.5.1.1.1 Hematological Toxicity Management

8.5.1.1.1.1 Patients with grade 2 or 3 hematological toxicity will not receive the subsequent dose of vaccine until there is normalization of cbc parameters, as required per eligibility criteria.

8.5.1.1.1.2 In case of grade 2 or 3 hematological toxicity detected any time during protocol therapy, CBC with differential will be checked on a weekly basis.

8.5.1.1.1.3 If the subsequent vaccine dose needs to be postponed for more than 2 weeks, patients will be removed from protocol therapy.

8.5.1.1.1.4 Patients with grade 4 hematological toxicity at any time will be permanently removed from protocol therapy.

8.5.1.1.2 Non-Hematological Toxicity Management

8.5.1.1.2.1 Patients with grade 2 or 3 non-hematological toxicity will not have the subsequent dose of vaccine administered until the toxicity ameliorates down to a grade 1 level.

8.5.1.1.2.2 Patients will be seen twice a week with study physician visits, if a grade 3 non-hematological toxicity occurs.

8.5.1.1.2.3 If the subsequent dose of vaccine needs to be postponed for more than 2 weeks, patients will be permanently removed from protocol therapy.

8.5.1.1.2.4 Patients with grade 4 non-hematological toxicity at any time will be permanently removed from protocol therapy.

Patients may also be removed from protocol therapy at any time based on assessment of any other risks, at the discretion of the study investigators.

8.5.2 Disease Progression: Patients will be taken off-study if they have progressive disease (PD) or clinically significant deterioration at any time during the study if the investigator feels that (a) alternative prostate cancer therapy might benefit the patient, or (b) to continue on study might be unsafe for the patient. Patients receiving alternative prostate cancer therapies will still be followed for toxicity and immunologic evaluations.

8.5.3 Allergic Reactions: Patients will be removed from the study should they develop grade II allergic reactions.

8.5.4 Personal Reasons: As stated in the informed consent, patients may withdraw from the study at any time.

8.5.5 Clinical Judgment: A patient may be withdrawn from the study, if, in the opinion of the investigators, it is not in the patient's best interest to continue (e.g. an adverse experience, intercurrent illness, etc.)

8.5.6 The date of discontinuation and the reason(s) for patient discontinuation from the study will be recorded in the CRF. All evaluations that are required at the follow-up must be conducted for each patient who discontinues research intervention, regardless of the reason.

Regulatory and Reporting Requirements

The Data and Safety Monitoring Committee (DSMC) of the Holden Comprehensive Cancer Center will provide data and safety monitoring for this study. “The Data and Safety Monitoring Plan of the Holden Comprehensive Cancer Center” provides standard operating procedures to monitor all clinical cancer trials at the UIHC. All investigator-initiated trials are automatically monitored by the DSMC. A detailed data and safety monitoring plan for this study is on file with the DSMC and the Clinical Research Safety Officer (CRSO).

Data Management, Quality Control and Data Security

In order to protect confidentiality the subject will be assigned an identification number. This number will be used on all specimens from the subject and will be used for documentation purposes.

Data management for the optimal entry, processing, storage, and retrieval for this protocol's data will be accomplished by the principal investigator. The database will be located on a computer or in a locked cabinet in a locked office. This computer will be secured, accessible only by the research team. There will be more than one copy of the database. The second, secured, copy of the protocol data will be stored in a locked room accessible only by the research team. For quality control, auditing, and checking data for integrity, there will be a regular accounting of data periodically performed. The medical record and research record will be linked by the study identification number. The data managers in this trial will be responsible for verification of the accuracy of all data transferred from the medical record to the research record. These records will be audited quarterly by the Data Safety Monitoring Committee. All Data Safety Monitoring Committee reports will be provided to the USAMRMC Office of Research Protections, Human Research Protection Office as they become available.

Data will be kept on file on each patient for at least two years past the termination of that patient’s participation in the trial. After that period of time the data in the research records will be shredded and the electronic database deleted.

Information from the medical records of patients referred by physicians outside the University of Iowa and the VA Medical Center required to determine eligibility for this protocol will be placed in a research folder, identified by the patient code and will be available only to members of the clinical trial team (identified on pages 28 and 29). The signed HIPAA forms will be kept in the patient’s folder in the office of the referring physician. The records of such patients that are deemed ineligible following a screening procedure will have their signed Release of Information forms, informed consent document, and eligibility form kept in a research folder under similar security conditions. All other records for these ineligible patients will be shredded unless the information is required for ongoing medical care.

Table 6
Study Design and Testing

	Std. of Care or Res.	Prior to Study Entry	Day 1 First Inj.	After first 24 hrs.	30 d. 2nd Inj.	31 d.	44 d.	60 d. 3rd Inj.	61 d.	74 d.	90 d.	6 mo	9 mo	12 mo	Every 6 mo. to prog.	Annual to prog.
Immunization	R		X		X			X								
Physical Examination	S	X	X	X	X	X		X	X		X	X	X	X	X	
Performance Status	S	X			X			X			X	X	X	X	X	
Vital Signs	S	X	X	X	X	X		X	X		X	X	X	X	X	
Weight	S	X	X		X			X			X	X	X	X	X	
Blood for PSA	S/R	X			X (R)			X (R)			X	X	X	X	X	
anti-PSA Ab, and lymphocytes for cellular immunity	R	X	X		X		X	X		X	X	X	X	X	X	
CBC, differential	R	X		X	X			X			X	X	X	X	X	
AST, ALT, LDH, alkaline phosphatase, bilirubin	R	X		X	X			X			X					
Creatinine	R	X		X	X			X			X					
Urinalysis	R	X		X	X			X			X					
Chest x-ray	R	X[a]			X[a]			X[a]			X[a]	X[a]	X[a]	X[a]	X[a]	
Bone scan	S	X[b]						X[b]				X[b]		X[b]		X[b]
Abdominal/pelvic CT	S	X[c]										X[c]		X[c]		X[c]

S = Procedures considered “Standard of Care” for prostate cancer patients.
R = Procedures considered “Research” as part of this Clinical Trial.

[a] will be repeated only if abnormal at screening or if patient develops a fever.
[b] will be repeated only if he patient demonstrates a rise in PSA
[c] will be repeated only if abnormal at screening.

9 DRUG FORMULATION AND PROCUREMENT

9.1 Drug Name

Adenovirus/PSA (Ad/PSA)

9.2 Classification

Vaccine

9.3 Mode of Action

The adenovirus is a replication-deficient virus unable to produce virus progeny in the infected cells. The virus will infect cells in the location of the injection site, the PSA gene will produce the protein product which will be recognized as an antigen by the immune system and produce anti-PSA immune responses. Based upon our pre-clinical studies in an animal model of human prostate cancer, these responses, mainly the CD8+ CTL response, will cause the destruction of PSA-secreting prostate tumors.

9.4 Dose Specifics and Route of Administration

The route of injection, vehicles for the vaccine, and dose schedules have been outlined in Section 5.1 of this protocol.

9.5 Availability

Produced and provided by Molecular Medicine, LLC, San Diego, CA

9.6 Storage

The vaccine is stored in the UIHC's Pharmacy Department in a -70^{o}C temperature-monitored and controlled access freezer. Only the Investigational Pharmacist will remove the vaccine from the freezer and enter the amount removed in a trial-specific log.

9.7 Injection Procedures

At the weekly meetings of the clinical trial team at which the eligibility of the patients is decided, orders for the initial vaccine injection will be written by clinician members of the team. Orders for the second and third vaccine injections will be written at the meetings immediately prior to the date of the scheduled injections. On the designated days of injections and immediately prior to vaccination, the Investigational Pharmacist will mix the Ad/PSA (10^8 pfu or 4.4×10^9 particles) with the Gelfoam powder (30 mg/ml). Each vial of the vaccine contains twice the volume needed for each injection. One half of the vial will contain the proper pfu and particles required for the vaccination. The injection material will be taken from the pharmacy in a 1 ml. syringe with 25 gauge needle and presented to staff in the General Clinical Research Center. Each patient will be injected with approximately 0.125 ml. of the vaccine/Gelfoam material subcutaneously in the thigh. The exact volume of the vaccination mixture (Ad/PSA in Gelfoam) is not as important as the exact number of pfu or particles and that is controlled by using the

required volume from the dose vial. The empty syringe will be disposed of in a biohazard container.

During the 24 hour stay in the GCRC the staff in the clinic will monitor patients for signs of adverse events. Physical examinations, vital signs, and laboratory tests will be performed as delineated in Table 6 on page 23 will be taken. Based upon the adverse events observed in the Phase I study the GCRC staff will look for signs of inflammation at the injection site, fever, cold and flu-like symptoms, fatigue, and changes in the absolute neutrophil count (ANC). The decision to discharge the patient at the end of the 24 hour period will be responsibility of one of the physician investigators, Drs. Williams, Joudi, Vaena or Smith.

9.8 Manufacturing

9.8.1 The PSA cDNA provided by Donald Tindall, Mayo Clinic, Rochester, MN, was placed 3' to the CMV promoter in a shuttle vector containing Ad5 DNA. The sequence inserted was the pre-pro form of PSA described by Lundwall (71) that encodes 262 amino acids with a predicted molecular weight of 28.8 kDa. Using methods previously described (72), the shuttle vector and E1a-E1b deletion mutant Ad5 DNA were transfected into HEK 293 cells, and recombination between the DNA species was allowed to occur. The amplification and purification of Ad/PSA was performed by the University of Iowa Gene Transfer Vector Core as previously described (73). Ad/lacZ used as a control was also obtained from the Gene Transfer Vector Core and is previously described (74).

9.8.2 The Principal Investigator provided the Ad/PSA vaccine used for the pre-clinical studies to Molecular Medicine, LLC of San Diego, CA for the production of the clinical grade product. Information on the manufacturing of the GMP Vaccine by Molecular Medicine, LLC is found in the accompanying documents supplied by the company.

10 STATISTICAL CONSIDERATIONS

The ideal endpoint would be a clinical outcome that is of particular relevance to the patient such as increased time to tumor progression, increased time to death or reducing the proportion of death. This trial is using a surrogate endpoint as a substitute to the clinically meaningful outcome since the tumor cannot be accessed directly. The association between the surrogate and survival rate had not been clearly established by any phase I & II trial. The trial consists of using Ad/PSA vaccine administered in multiple injections to prostate cancer patients with hormone—refractory metastatic prostate cancer—with the goal to induce anti-PSA T cells responses. Three injections of equal dose are proposed. The previous phase I trial consisting of a single injection in men with hormone-refractory metastatic prostate cancer was able to induce anti-PSA T cells responses. The Phase I consisting of a single injection using dose escalation protocol of the vaccine in an aqueous or matrix delivery vehicle did not show any significant AE. Additional pre-clinical pharmacology /toxicology studies required by the FDA did not show any significant side effects using the three-injection schedule. The primary endpoint is the serum PSADT.

For efficacy purposes, we expect at least 50% of the patients to show 50% increase in PSADT. This proportion is judged clinically important and anything less than 30% can stop the trial for futility. The ideal design would be a two-stage design of Simon (1989) requiring 32

patients. After testing the research intervention on 12 patients, if 3 did not have a 50% increase in PSADT, the trial will be terminated. Otherwise, an additional 20 patients will be recruited for the trial. The expected sample size will be 19.73 and the probability of early termination, 0.72. If the research intervention is not effective, there is 0.1 probability of concluding that it is. If the research intervention is effective, there will be 0.2 probability of concluding that it is not. However, due to the complexity of the trial, we will not use Simon (1989) but a more conservative approach consisting of using 31 patients in a one-stage design at efficacy level and a two-stage design at toxicity level for stopping rules. By using a one-stage design at efficacy endpoint, we are making the probability of early termination for efficacy purposes to be zero. Early termination will be based on toxicity only. The reason for this design is due to the nature of the research intervention and is explained below. Enrollment will not stop unless stopping rules based on toxicity are satisfied:
"After the third vaccine dose is administered, toxicity will be evaluated 90 days into the trial"

Due to the nature of the research intervention, anti-tumor activity will be potentially delayed and the primary efficacy endpoint will be determined 18 to 20 months after initial patient accrual. Since we will be able to assess PSADT after nearly 20 months into the study, it will be unreasonable to stop the study due to unsatisfactory results prior to that point. This is the main reason why we will carry a one-stage design for the primary endpoint and a two-stage design for toxicity. Criteria for proceeding with enrollment into a subsequent stage prior to the two-year efficacy evaluation will be based on assessment of toxicity in the patient cohorts since this can be done as early as 90 days into the trial.

For toxicity purposes, we will test the null hypothesis that the toxicity level is less than 15% versus the alternative that it is greater than 35%. The trial will be stopped whenever the null hypothesis is rejected. We will conduct these tests in 2 different looks—one after an enrolment of 17 subjects and the second after 25 subjects are enrolled.

If 5 out of 17 show grade 3 toxicity or higher, the trial will stop (this will correspond to testing the toxicity hypotheses at level alpha=0.05 with a power 77%); otherwise, an additional 8 subjects will enter the trial. If 7 out of 25 show a grade 3 toxicity or higher, the trial will stop (this will be equivalent to testing the toxicity hypotheses at level alpha =0.05 with a power 82%); otherwise we will proceed to full registration. The overall type I error for this test is 0.09.

The reasons for using different looks at toxicity level is because we are using a surrogate endpoints that had not been proven formerly to have an ease of predictability of an outcome of direct relevance to patients; also, it is not quite clear how theses surrogates relate to the pathway of the natural disease and to overall survival rate.

Simon, Richard. "Optimal Two-Stage Designs for Phase II Clinical Trials," Controlled Clinical Trials, 1989, Volume 10, pages 1-10.
De Gruttola Victor et Al., "Considerations in the Evaluation of Surrogate Endpoints in Clinical Trials: Summary of NIH workshop" Controlled Clinical Trials, 2001, Volume 22, pages 485-502

Addendum
PHASE II STUDY OF ADENOVIRUS/PSA VACCINE IN MEN WITH HORMONE - REFRACTORY PROSTATE CANCER
Food and Drug Administration (FDA) Investigational New Drug (IND) #9706
Department of Defense, Prostate Cancer Research Program A-14059.2

The following are reporting requirements and responsibilities of the Principal Investigator to the United States Army Medical Research and Materiel Command's (USAMRMC) Office of Research Protections (ORP), Human Research Protection Office (HRPO).

1. The protocol will be conducted in accordance with the protocol submitted to and approved by the USAMRMC ORP HRPO and will not be initiated until written notification of approval of the research project is issued by the USAMRMC ORP HRPO.

2. Accurate and complete study records will be maintained and made available to representatives of the U.S. Army Medical Research and Materiel Command as a part of their responsibility to protect human subjects in research. Research records will be stored in a confidential manner so as to protect the confidentiality of subject information.

3. All unanticipated problems involving risk to subjects or others, serious adverse events related to participation in the study and subject deaths related to participation in the study should be promptly reported by phone (301-619-2165), by email (hsrrb@det.amedd.army.mil), or by facsimile (301-619-7803) to the USAMRMC, Office of Research Protections, Human Research Protection Office. A complete written report will follow the initial notification. In addition to the methods above, the complete report will be sent to the U.S. Army Medical Research and Materiel Command, ATTN: MCMR-ZB-PH, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

4. Any deviation to the protocol that may have an effect on the safety or rights of the subject or the integrity of the study must be reported to the USAMRMC ORP HRPO as soon as the deviation is identified.

5. Major modifications to the research protocol and any modifications that could potentially increase risk to subjects must be submitted to the USAMRMC ORP HRPO for approval prior to implementation. All other amendments will be submitted with the continuing review report to the USAMRMC ORP HRPO for acceptance.

6. A copy of the approved continuing review report and the local IRB approval notification will be submitted to the USAMRMC ORP HRPO as soon as these documents become available. A copy of the approved final study report and local IRB approval notification will be submitted to the USAMRMC ORP HRPO as soon as these documents become available.

7. The knowledge of any pending compliance inspection/visit by the FDA, OHRP, or other government agency concerning clinical investigation or research, the issuance of Inspection Reports, FDA Form 483, warning letters or actions taken by any Regulatory Agencies including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements will be reported immediately to USAMRMC ORP HRPO. "

Roles and Responsibilities of Study Personnel:

David M. Lubaroff, PhD, Principal Investigator – Dr. Lubaroff, along with Dr. Williams, will manage all aspects of the trial, from assisting in patient recruitment, co-chairing the clinical trial meetings, to supervising the immunologic testing of the patients' sera and lymphocytes for anti-PSA immune responses. He will work together with Dr. Williams the Co-Principal Investigator on all important clinical issues for the trial.

Richard D. Williams, MD; Co-Principal Investigator – Dr. Williams will manage all patient care activities associated with this proposal and will work together with Dr. Lubaroff on managing the trial. Dr. Williams will function as co-chair of the weekly trial team meetings. He will be a major participant in the clinical management of prostate cancer patients that includes recruitment, patient management, assessing clinical response and any adverse events and long-term follow-up during the trial.

Fadi Joudi, MD, Co-Investigator – Dr. Joudi, as the second of two clinical urologists on the clinical trial team, will also participate in the recruitment and clinical management of the patients. As Chief of Urology at the Iowa City Veterans Affairs Medical Center (ICVAMC) Dr. Joudi will be an active participant in the treatment and follow-up of patients at that hospital. He will work closely with Dr. Vaena in that capacity.

Daniel Vaena, MD, Co-Investigator – Dr. Vaena is a Medical Oncologist who cares for prostate cancer patients following recurrences of their disease. He will assist in the recruitment and care of patients in the trial. As an attending oncologist at the Iowa City Veterans Affairs Medical Center (ICVAMC) Dr. Vaena will be an active participant in the treatment and follow-up of patients at that hospital. He will work closely with Dr. Joudi in that capacity.

Mark C. Smith, MD, Co-Investigator – Dr. Smith is a Radiation Oncologist who is responsible for radiation therapy of men with prostate cancer. He will assist in the recruitment and care of patients in the trial.

Tammy Madsen, BA, MPAS – **Study Coordinator** - Ms. Madsen, a Physician's Assistant in the Department of Urology, will be the study coordinator for the trial, and will coordinate activities with all of the clinical trial team, administer the informed consent document and obtain patient written consent, coordinate participant accrual, administer the vaccine to patients, and participate in the follow-up examinations.

Carlene Etscheidt, BSN, MSN – Clinical Trial Coordinator – Ms. Etscheidt participates in clinical trials in the Holden Comprehensive Cancer Center at the University of Iowa. She has and will continue to guide the clinical protocol through the institutional and federal other regulatory approval processes. She will also work closely with Ms. Madsen in follow-up visits of the patients.

Pamela Zehr, BSN, MSN - Clinical Trial Coordinator – Ms. Zehr also participates in clinical trials in the Holden Comprehensive Cancer Center at the University of Iowa. She will act as a backup for Ms. Etscheidt and also work closely with Ms. Madsen in patient follow-up.

Sara Miller, RN - Study Coordinator VAMC – Ms. Miller will be responsible for trial for patients enrolled in the trial at the VA Medical Center. She will work closely with all members of the trial team and attend the weekly meetings to discuss eligibility and follow-up of patients.

Gideon Zamba, PhD – Biostatistician – Dr. Zamba participated in the discussions pertinent to the development of the clinical protocols, performed the power analysis, and constructed the section on statistical considerations for the trial. He will assist in the data analysis and statistical interpretation of the patient data.

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INFORMED CONSENT DOCUMENT
FDA IND #9706
DOD HSRRB #A14059.1

Project Title: **Phase II study of adenovirus/PSA vaccine in men with recurrent prostate cancer after local therapy**

Research Team: David M. Lubaroff, PhD; Richard D. Williams, MD; Fadi Joudi, MD; Daniel Vaena, MD; Mark C. Smith, MD, Tammy Madsen PAC; Carlene Etscheidt, BSN, MSN, Pamela Zehr, BSN, MA; Sara Miller, BSN

This consent form describes the research study to help you decide if you want to participate. This form provides important information about what you will be asked to do during the study, about the risks and benefits of the study, and about your rights as a research subject.

- If you have any questions about or do not understand something in this form, you should ask the research team for more information.
- You should discuss your participation with anyone you choose such as family or friends.
- Do not agree to participate in this study unless the research team has answered your questions and you decide that you want to be part of this study.

WHAT IS THE PURPOSE OF THIS STUDY?

This is a research study. We are inviting you to participate in this research study because you have recurrent cancer of the prostate. This study involves a research intervention with an Ad/PSA vaccine. This is a virus vaccine in which the gene for prostate specific antigen (PSA) has been placed into a common cold virus termed adenovirus (Ad) to produce this Ad/PSA product. Adenovirus is a common virus found in human respiratory systems. In its normal state, it can reproduce and cause a respiratory infection. Respiratory illnesses caused by adenovirus infections range from the common cold to pneumonia, croup and bronchitis. The adenovirus used in this research study will not be infectious, but may still cause flu or cold like symptoms. PSA is produced by normal and cancerous prostate cells. Since you have previously been treated for your prostate cancer by surgery or irradiation the only cells that will be secreting PSA are the remaining cancer cells.

The purpose of this study is to determine whether vaccination with the Ad/PSA prostate cancer vaccine will activate the immune system against PSA, which might have a positive effect on prostate cancer. Other scientists have also been working on cancer vaccines and, although many show promising results in pre-clinical studies and are in Phase II and Phase III clinical trials, none have been approved for therapeutic use.

The Ad/PSA vaccine is considered investigational, which means that it has not been approved by the U.S. Food and Drug Administration.

HOW MANY PEOPLE WILL PARTICIPATE?

Approximately 50 people will take part in this study at the University of Iowa and the Iowa City Veterans Affairs Medical Center.

HOW LONG WILL I BE IN THIS STUDY?

If you agree to take part in this study, your involvement will last for approximately 2 years, perhaps longer. Much depends upon the effect of the vaccine on your cancer. You will receive a total of three vaccinations. After each of the vaccinations, you will be asked to stay in our General Clinical Research Center (GCRC) for 24 hours so we can monitor how you respond to the vaccination. You will be asked to return to the clinic at regularly scheduled times after the vaccinations to undergo a physical examination and blood tests to evaluate whether the vaccine is causing any side effects. On some visits you will also have x-rays and scans to monitor the progress of your prostate cancer following the vaccinations**.** After the first vaccination you will have visits on days 30 (vaccination 2), 44, 60 (vaccination 3), 74, 90, and 6, 9, and 12 months**.** Depending upon how you respond to the vaccination you will be asked to return every six months after the 12 month visit and have the same examinations and blood tests. These visits will continue until you show progression of your prostate cancer.

WHAT WILL HAPPEN DURING THIS STUDY?

If you are interested in participating in this study, you will first be asked to sign this informed consent document before any research specific testing is initiated. Once the consent has been signed, you will be scheduled to complete tests and procedures to determine if you are eligible to participate in this study. By signing this Informed Consent document you grant permission for the study investigators to access your medical records for the purposes of evaluating your eligibility for entrance into this vaccine trial. If you are eligible and would like to participate, you will be randomly placed into one of two research interventions – Arm A or Arm B. In Arm A, you would receive the vaccination once every 30 days for a total of three vaccinations. In Arm B, you would be asked to start on androgen deprivation therapy 14 days before receiving the three monthly vaccinations.

Listed are the tests and procedures that take place for screening and at the study visits.
SCREENING VISIT:

- Physical exam
- Chest X-ray
- Abdominal/pelvic CT
- Bone scan
- Blood tests*
- Urine tests

VISIT 1 (first vaccination and 24 hours stay in GCRC):

- Physical exam
- Blood tests*
- Urine tests

VISIT 2 (second vaccination and 24 hours stay in GCRC):

- Physical exam
- Blood tests*
- Urine tests
- Chest X-ray[a]

VISITS 3 AND 5 (Days 44 and 74):

- Physical exam

- Blood tests*
- Urine tests

VISIT 4 (third vaccination and 24 hours stay in GCRC):

- Physical exam
- Blood tests*
- Urine tests
- Chest X-ray[a]
- Abdominal/pelvic CT[c]
- Bone scan[b]

VISITS 6 and 8 (Day 90 and 9 months):

- Physical exam
- Blood tests*
- Urine tests
- Chest X-ray[a]

VISITS 7, 9 (6 and 12 months) AND ALL VISITS AFTER 1 YEAR (every 6 months until completion of study):

- Physical exam
- Blood tests*
- Urine tests
- Chest X-ray[a]
- Abdominal/pelvic CT[c]
- Bone scan[b]

*4 tablespoons of blood will be obtained at each time point for all clinical and research testing of blood counts, liver function, and kidney function.
[a] Will be performed only if the results are abnormal at screening or if a fever develops.
[b] Will be performed only if there is a rise in PSA.
[c] Will be performed only if the results are abnormal at screening.

PSA measurements, CT and bone scans are routinely used to follow disease recurrence and/or progression in individual prostate cancer patients and are considered standard of care. Laboratory measurements such as blood counts, liver function and kidney function tests, are routinely used to follow the health of a prostate cancer patient; however, they would not normally be performed as often as they will in this trial to assess possible vaccination toxicities. Therefore, they would be considered part of the research protocol.

WHAT ARE THE RISKS OF THIS STUDY?

VACCINE:

There may be some risks from being in this research study. This study uses a form of gene transfer. Since gene transfer is a new method of treating disease not all of the risks associated with this research intervention are known. In a previous study using adenoviruses, a death did occur. In 1999 a young man died from liver failure when a very high dose of an adenovirus was injected directly into the vein leading to the liver. However, results from our first study of the vaccine indicated that none of the

subjects suffered any severe vaccine-related side effects when the vaccine was injected subcutaneously. However, the first study used a single vaccination whereas this study will use three vaccinations. Pre-clinical studies in laboratory animals did not produce any adverse events using the three injection schedule to be used in this clinical study. Based upon our first study, the adenovirus vaccine may cause a local redness and swelling at the site of vaccination, some flu or cold like symptoms, a decrease in white blood cell count (which could result in an increased risk of infection), temporary leakage of protein into your urine, headaches, or fever. Temporary leakage of protein into the urine, which in small amounts does not cause any adverse effects. In large amounts, it can cause swelling and kidney damage in the short or long term. However, the chances of large amounts of protein leakage are very small. If you experience discomfort as a result of the vaccinations you may take over the counter pain relief such as aspirin, Tylenol, or ibuprofen. Do not use any oral steroids such as prednisone as this will depress your ability to develop immune responses to the PSA and compromise the research study.

The vaccine will be mixed with collagen and injected into your thigh. Although there were no additional side effects when the vaccine was administered in collagen as compared to the vaccine alone in the first study, there is the possibility of infection, fluid accumulation or bruising at the injection site. If this occurs you may take the same over the counter medications listed above that will ease your discomfort and lower any fever that may occur, but do not take oral steroids.

There may be some local tenderness, reddening, or swelling at the vaccination site, but this will normally disappear within a few days. The PSA vaccine may induce antibodies against PSA protein. This could interfere with the ability of your health care provider to monitor your PSA blood levels. The antibodies to PSA may lower the amount of the PSA protein in your blood that would not necessarily reflect a clinical change in your cancer. For example, the anti-PSA antibodies may attach to the PSA in your blood and that would not permit the PSA to be measured by the current tests. We are working with other scientists to study this possibility. We are not certain how long the potential for lowering your serum PSA levels will last during the study. However, other methods of follow-up include physical examination, imaging studies (for example, CT, MRI, bone scan), and selected laboratory studies such as alkaline phosphatase will still be useful to follow your disease. Results of these tests will be included in a letter to your local referring physician.

The Ad/PSA vaccine will be injected in a collagen Gelfoam matrix, a mixture that has been shown in our pre-clinical research to enhance the production of immune responses to the PSA protein. Gelfoam is a product derived from pigs. If you object to the use of, or are allergic to, a pork product you have the right to decline participation in this study.

Your physician will be checking you closely to see if any side effects are occurring. Routine blood and urine tests will be done to monitor the effects of the investigational vaccine. Many side effects disappear after the drug is stopped. In the meantime, your health care provider may prescribe medications to keep these side effects under control.

There is no need for the use of contraceptive devices following injection of the adenovirus/PSA vaccine since the virus will not be present in the semen and cannot be transmitted to your partner.

The following summarizes the known risks of the Ad/PSA vaccine. There is still very limited experience with the vaccine and there could be other side effects we do not yet know about. As with any investigational product, there is always some chance of unexpected serious or even life-threatening side effects.

Likely/Common Risks (more than 35%)
Life Threatening – none
Serious – none
Mild – local tenderness, reddening, swelling at vaccination site

Less Likely/Less Common (10% - 35%)
Life Threatening – none
Serious – none
Mild – none

Rare (less than 10%)
Life Threatening – none
Serious – decrease in white blood cell count
Mild – flu or cold-like symptoms
protein leakage into urine
headache
fever

ANDROGEN DEPRIVATION (HORMONE) THERAPY:

If you are in the group of subjects that will also receive androgen deprivation therapy, there are some side effects associated with this intervention. Common side effects include fluid retention, hair loss, hot flashes, nausea, vomiting, bone pain, memory changes, and depression. These side effects are not expected from the vaccine and should not interfere with the identification of vaccine-associated side effects. However, you will be closely monitored by the research team for the development of hormone-related side effects. If side effects occur, investigators will take necessary steps to treat them, including discontinuation of the medication and/or terminating your participation in the study.

Likely/Common (more than 35%)
Like Threatening - none
Serious – bone calcium loss
Mild – hot flashes

Less Likely/Less Common (10% - 35%)
Life Threatening - none
Serious - none
Mild – hair loss, fluid retention

Rare (less than 10%)
Like Threatening - none
Serious – nausea, vomiting, bone pain, memory changes
Mild - depression

BLOOD DRAW

During the screening for eligibility and all follow up visits you will have blood drawn for testing. This may cause bruising or infection at the needle site and/or fainting. We will attempt to minimize these possible side effects by using a trained professional for all blood draws.

Likely/Common Risks (more than 35%)
Life Threatening – none
Serious – none
Mild – local tenderness, reddening, swelling at blood draw site

Less Likely/Less Common (10% - 35%)
Life Threatening – none
Serious – none
Mild – none

Rare (less than 10%)
Life Threatening – none
Serious – none
Mild – none

ARE THERE ANY UNFORESEEN RISKS?

In addition to the risks described above, there may be unknown risks, or risks that we did not anticipate, associated with being in this study.

WHAT ARE THE BENEFITS OF THIS STUDY?

We don't know if you will benefit from being in this study. However, we hope that, in the future, other people might benefit from this study as data obtained from this study may indicate the level of benefit obtained from the vaccination.

WHAT OTHER TREATMENT OPTIONS ARE THERE?

Before you decide whether or not to be in this study, the study physician will discuss the other options that are available to you. Instead of being in this study, alternatives which could be considered in your case include watchful waiting or hormonal therapy, if you are not already on this treatment. Your study health care provider can provide detailed information about your disease and the benefits of hormonal therapy. You should feel free to discuss your disease and prognosis with your health care provider.

WILL IT COST ME ANYTHING TO BE IN THIS STUDY?

There may be additional costs for being on this study. These include time lost at work and travel expenses. The vaccine Ad/PSA, the costs of administration, and your stay in the GCRC will be provided to you without cost. You and/or your insurance company will not be billed for any procedures related specifically to this study.

You and/or your medical/hospital insurance carrier will remain responsible for your regular medical care expenses.

WILL I BE PAID FOR PARTICIPATING?

Other than medical care that may be provided and any other payment specifically stated in the consent form, there is no other compensation available for your participation in this research.

WHO IS FUNDING THIS STUDY?

The U.S. Department of Defense (DOD) is funding this research study. This means that the University of Iowa is receiving payments from the DOD to support the activities that are required to conduct the study. No one on the research team will receive a direct payment or increase in salary from the DOD for conducting this study.

WHAT IF I AM INJURED AS A RESULT OF THIS STUDY?

- If you are injured or become ill from taking part in this study, medical treatment is available at the University of Iowa Hospitals and Clinics.
- No compensation for treatment of research-related illness or injury is available from the University of Iowa unless it is proven to be the direct result of negligence by a University employee.
- If you experience a research-related illness or injury, you and/or your medical or hospital insurance carrier will be responsible for the cost of treatment.
- If you are hurt or get sick because of this research study, you can also receive medical care at an Army hospital or clinic free of charge. You will only be treated for injuries that are directly caused by the research study. The Army will not pay for your transportation to and from the hospital or clinic. If you have questions about this medical care, talk to the principal investigators for this study, (David M. Lubaroff, PhD, 319-335-8423 or Richard D. Williams, MD, 319-356-0760). If you pay out-of-pocket for medical care elsewhere for injuries caused by this research study, contact Drs Lubaroff or Williams. If the issue cannot be resolved, contact the U.S. Army Medical Research and Materiel Command (USAMRMC) Office of the Staff Judge Advocate (legal office) at (301) 619-7663/2221.
- In the event of an emergency you should seek immediate care by calling 911, your local physician, the University of Iowa (319-356-1616) or Iowa City VA Medical Center urology staff on call (319-338-0581).

WHAT ABOUT CONFIDENTIALITY?

We will keep your participation in this research study confidential to the extent permitted by law. However, it is possible that other people may become aware of your participation in this study. For example, federal government regulatory agencies, the National Institutes of Health/Office of Biologic Activities (NIH/OBA), the Department of Defense, the U.S. Food and Drug Administration, auditing departments from the University of Iowa, and the University of Iowa Institutional Review Board (a committee that reviews and approves research studies) may inspect and copy records pertaining to this research. Some of these records could contain information that personally identifies you.

In the future, the granting agency may continue to use your health information that is collected as part of this study. For example, they may combine information from this study with the results of other studies to re-analyze the safety and effectiveness of the study medication, to evaluate other products or therapies, to develop a better understanding of a disease, or to improve the design of future research studies. The funding agency may also share information from this study with regulatory agencies in foreign countries.

To help protect your confidentiality, we will assign a specific coded number to your file that will appear

on the data forms and files, all written files will be kept in a locked office and information on computers will be protected by secure passwords. Only your University of Iowa Hospitals and Clinics or VA Medical Center records will contain information that associates your name with the research study. A sample of your blood will be sent to the Mayo Clinic for special laboratory testing. These blood samples will only be identified by a coded trial specific code. Only the University of Iowa and VA Medical Center investigators will know your identity and will not disclose that information to our Mayo Clinic colleagues. If we write a report or article about this study or share the study data set with others, we will do so in such a way that you cannot be directly identified.

A copy of the Informed Consent Document will be placed in your medical record.

WILL MY HEALTH INFORMATION BE USED DURING THIS STUDY?

The Federal Health Insurance Portability and Accountability Act (HIPAA) requires the University of Iowa Health Care or the Iowa City VA Medical Center to obtain your permission for the research team to access or create "protected health information" about you for purposes of this research study. Protected health information is information that personally identifies you and relates to your past, present, or future physical or mental health condition or care. We will access or create health information about you, as described in this document, for purposes of this research study and for your treatment. Once the University of Iowa Health Care or Iowa City VA Medical Center has disclosed your protected health information to us, it may no longer be protected by the Federal HIPAA privacy regulations, but we will continue to protect your confidentiality as described under "Confidentiality."

We may share your health information related to this study with other parties including federal government regulatory agencies, the NIH/OBA, the DOD, the University of Iowa Institutional Review Boards and support staff.

You cannot participate in this study unless you permit us to use your protected health information. If you choose *not* to allow us to use your protected health information, we will discuss any non-research alternatives available to you. Your decision will not affect your right to medical care that is not research-related. Your signature on this Consent Document authorizes the University of Iowa Health Care to give us permission to use or create health information about you.

Although you may not be allowed to see study information until after this study is over, you may be given access to your health care records by contacting your health care provider. Your permission for us to access or create protected health information about you for purposes of this study has no expiration date. You may withdraw your permission for us to use your health information for this research study by sending a written notice to David M. Lubaroff, PhD, Department of Urology, 200 Hawkins Drive, Iowa City, IA 52242. However, we may still use your health information that was collected before withdrawing your permission. Also, if we have sent your health information to a third party, such as the study sponsor, or we have removed your identifying information, it may not be possible to prevent its future use. You will receive a copy of this signed document.

IS BEING IN THIS STUDY VOLUNTARY?

Taking part in this research study is completely voluntary. You may choose not to take part at all. If you decide to be in this study, you may stop participating at any time. If you decide not to be in this study, or if you stop participating at any time, you won't be penalized or lose any benefits for which you otherwise qualify.

WHAT IF I DECIDE TO DROP OUT OF THE STUDY?

If you decide to stop participating in the study, we would like to continue to obtain follow-up information about your health through contact with your other physicians and/or periodic phone calls to you from our research staff, unless you notify us otherwise. An important follow-up visit will be 30 days after your last injection. Even if you decide not to continue participating in the study we would like you to keep this appointment.

WILL I RECEIVE NEW INFORMATION ABOUT THE STUDY WHILE PARTICIPATING?

If we obtain any new information during this study that might affect your willingness to continue participating in the study or directly affect your continued health, we will promptly provide you with that information.

CAN SOMEONE ELSE END MY PARTICIPATION IN THIS STUDY?

Under certain circumstances, the researchers or the study sponsor might decide to end your participation in this research study earlier than planned. This might happen for any one or more of the following reasons: (a) in our judgment it would not be safe for you to continue, (b) because your condition has become worse, or (c) because funding for the research study has ended.

WHAT IF I HAVE QUESTIONS?

We encourage you to ask questions. If you have any questions about the research study itself, please contact: David Lubaroff at (319) 335-8425 or Richard D. Williams, MD at (319) 356-0760 or Dr. Fadi Joudi at (319) 384-5993 of the Department of Urology, or Dr. Daniel Vaena (319) 356-1616 of the Department of Internal Medicine Hematology/Oncology. If you are calling after hours please call 319-356-1616, ask for the Urology Resident or Oncology Fellow on call and tell the operator you are a research subject.

If you have questions, concerns, complaints about your rights as a research subject or about research related injury, please contact the Human Subjects Office, 340 College of Medicine Administration Building, The University of Iowa, Iowa City, Iowa, 52242, (319) 335-6564, or e-mail irb@uiowa.edu. General information about being a research subject can be found by clicking “Info for Public” on the Human Subjects Office web site, http://research.uiowa.edu/hso.

FOR IRB USE ONLY
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This Informed Consent Document is not a contract. It is a written explanation of what will happen during the study if you decide to participate. You are not waiving any legal rights by signing this Informed Consent Document. Your signature indicates that this research study has been explained to you, that your questions have been answered, and that you agree to take part in this study. You will receive a copy of this form.

Subject's Name (printed): _____

Do not sign this form if today's date is on or after $STAMP_EXP_DT.

_____ _____
(Signature of Subject) (Date)

Permanent Address (printed): _____

_____

Statement of Person Who Obtained Consent

I have discussed the above points with the subject or, where appropriate, with the subject's legally authorized representative. It is my opinion that the subject understands the risks, benefits, and procedures involved with participation in this research study.

_____ _____
(Signature of Person who Obtained Consent) (Date)

INFORMED CONSENT DOCUMENT
FDA IND #9706
DOD HSRRB #A14059.2

Project Title: **Phase II study of Adenovirus/PSA vaccine in men with hormone - refractory prostate cancer**

Research Team: David M. Lubaroff, PhD; Richard D. Williams, MD; Fadi Joudi, MD; Daniel Vaena, MD; Mark C. Smith, MD, Tammy Madsen PA; Carlene Etscheidt, BSN, MSN; Pamela Zehr, BSN, MA; Sara Miller, BSN

This consent form describes the research study to help you decide if you want to participate. This form provides important information about what you will be asked to do during the study, about the risks and benefits of the study, and about your rights as a research subject.

- If you have any questions about or do not understand something in this form, you should ask the research team for more information.
- You should discuss you participation with anyone you choose such as family or friends.
- Do not agree to participate in this study unless the research team has answered your questions and you decide that you want to be part of this study.
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WHAT IS THE PURPOSE OF THIS STUDY?

This is a research study. We are inviting you to participate in this research study because you have hormone refractory cancer of the prostate, that is, your prostate cancer is no longer responding to hormone therapy. This study involves a research intervention with an Ad/PSA vaccine. This is a virus vaccine in which the gene for prostate specific antigen (PSA) has been placed into a common cold virus termed adenovirus (Ad) to produce this Ad/PSA product. Adenovirus is a common virus found in human respiratory systems. In its normal state, it can reproduce and cause a respiratory infection. Respiratory illnesses caused by adenovirus infections range from the common cold to pneumonia, croup and bronchitis. The adenovirus used in this research study will not be infectious, but may still cause flu or cold like symptoms. PSA is produced by normal and cancerous prostate cells. Since you may have previously been treated for your prostate cancer by surgery or irradiation the only cells that will be secreting PSA are the remaining cancer cells.

The purpose of this study is to determine whether vaccination with the Ad/PSA prostate cancer vaccine will activate your immune system against PSA, which might have a positive effect on prostate cancer. Other scientists have also been working on cancer vaccines and, although many show promising results in pre-clinical studies and are in Phase II and Phase III clinical trials, none have been approved for therapeutic use.

The Ad/PSA vaccine is considered investigational, which means that it has not been approved by the U.S. Food and Drug Administation.

HOW MANY PEOPLE WILL PARTICIPATE?

Approximately 32 people will take part in this study at the University of Iowa and the Iowa City Veterans Affairs Medical Center.

HOW LONG WILL I BE IN THIS STUDY?

If you agree to take part in this study, your involvement will last for approximately 2 years, perhaps longer. Much depends upon the effect of the vaccine on your cancer. You will receive a total of three vaccinations. After each of the vaccinations, you will be asked to stay in our General Clinical Research Center (GCRC) for 24 hours so we can monitor how you respond to the vaccination. You will be asked to return to the clinic at regularly scheduled times after the vaccination to undergo a physical examination and blood tests to evaluate whether the vaccine is causing any side effects. On some visits you will also have x-rays and scans to follow the progress of your prostate cancer follwing the vaccinations. Following the first vaccinations you will have visits on days 30 (vaccination 2), 44, 60 (vaccination 3), 74, 90, and 6, 9, and 12 months. Depending upon how you respond to the vaccination you will be asked to return every six months after the 12 month visit and have the same examinations and blood tests. These visits will continue until you show progression of your prostate cancer.

WHAT WILL HAPPEN DURING THIS STUDY?

If you are interested in participating in this study, you will first be asked to sign this informed consent document before any research specific testing is initiated. Once the consent has been signed, you will be scheduled to complete tests and procedure to determine if you are eligible to participate in this study. By signing this Informed Consent document you grant permission for the study investigators to access your medical records for the purposes of evaluating your eligibility for entrance into this vaccine trial. If you are eligible and wish to participate, you will receive the vaccine once every 30 days for a total of three vaccinations. Listed below are the tests and procedures that take place for screening and at the study visits.

SCREENING VISIT:

- Physical exam
- Chest X-ray
- Abdominal/pelvic CT
- Bone scan
- Blood tests*
- Urine tests

VISIT 1 (first vaccination and 24 hour stay in GCRC):

- Physical exam
- Blood tests*
- Urine tests

VISIT 2 (second vaccination and 24 hour stay in GCRC):

- Physical exam
- Blood tests+
- Urine test*
- Chest X-ray[a]

VISITS 3 AND 5 (Days 44 and 74):

- Physical exam
- Blood tests*
- Urine tests

VISIT 4 (third vaccination and 24 hour stay in GCRC):

- Physical exam
- Blood tests*
- Urine tests
- Chest X-ray[a]
- Abdominal/pelvic CT[c]
- Bone scan[b]

VISITS 6 and 8 (Day 90 and 9 months):

- Physical exam
- Blood tests*
- Urine tests
- Chest X-ray[a]
-

VISITS 7, 9 (6 and 12 months) AND ALL VISITS AFTER 1 YEAR (every 6 months until completion of study:

- Physical exam
- Blood tests*
- Urine tests
- Chest x-ray[a]
- Abdominal/pelvic CT[c]
- Bone scan[b]

* 4 tablespoons of blood will be obtained at each time point for all clinical and research testing of blood counts, liver function, and kidney function.
[a] Will be performed only if the results are abnormal at screening or if a fever develops.
[b] Will be performed only if there is a rise in PSA.
[c] Will be performed only if the results are abnormal at screening.

PSA measurements, CT and bone scans would routinely be used to follow your disease and would be considered standard of care. Laboratory measurements such as blood tests are also used to follow the health of a prostate cancer patient, but if you are in this research study, these tests will be done more often than they normally would. Therefore, they would be considered part of the research protocol rather than standard of care.

WHAT ARE THE RISKS OF THIS STUDY?

There may be some risks from being in this research study. This study uses a form of gene transfer. Since gene transfer is a new method of treating disease not all of the risks associated with this research intervention are known. In a previous study using adenoviruses, a death did occur. In 1999 a young man died from liver failure when a very high dose of an adenovirus was injected directly into the vein leading to the liver. However, results from our first study of the vaccine indicated that none of the subjects suffered any severe vaccine-related side effects when the vaccine was injected subcutaneously. However, the first study used a single vaccination whereas this study will use three vaccinations. Pre-clinical studies in laboratory animals did not produce any adverse events using the three injection schedule to be used in this clinical study. Based upon our first study, the adenovirus vaccine may cause a local redness and swelling at the site of vaccination, some flu or cold like symptoms, a decrease in white blood cell count (which could result in an increased risk of infection), temporary leakage of protein into your urine, headaches, or fever. Temporary leakage of protein into

the urine, which in small amounts does not cause any adverse effects. In large amounts, it can cause swelling and kidney damage in the short or long term. However, the chances of large amounts of protein leakage are very small. If you experience discomfort as a result of the vaccinations you may take over the counter pain relief such as aspirin, Tylenol, or ibuprofen. Do not use any oral steroids such as prednisone as this will depress your ability to develop immune responses to the PSA and compromise the research study.

The vaccine will be mixed with collagen and injected into your thigh. Although there were no additional side effects when the vaccine was administered in collagen as compared to the vaccine alone in the first study, there is the possibility of infection, fluid accumulation or bruising at the injection site. If this occurs you may take the same over the counter medications listed above that will ease your discomfort and lower any fever that may occur, but do not take oral steroids.

There may be some local tenderness, reddening, or swelling at the vaccination site, but this will normally disappear within a few days. The PSA vaccine may induce antibodies against PSA protein. This could interfere with the ability of your health care provider to monitor your PSA blood levels. The antibodies to PSA may lower the amount of the PSA protein in your blood that would not necessarily reflect a clinical change in your cancer. For example, the anti-PSA antibodies may attach to the PSA in your blood and that would not permit the PSA to be measured by the current tests. We are working with other scientists to study this possibility. We are not certain how long the potential for lowering your serum PSA levels will last during the study. However, other methods of follow-up include physical examination, imaging studies (for example, CT, MRI, bone scan), and selected laboratory studies such as alkaline phosphatase will still be useful to follow your disease. Results of these tests will be included in a letter to your local referring physician.

The Ad/PSA vaccine will be injected in a collagen Gelfoam matrix, a mixture that has been shown in our pre-clinical research to enhance the production of immune responses to the PSA protein. Gelfoam is a product derived from pigs. If you object to the use of, or are allergic to, a pork product you have the right to decline participation in this study.

Your physician will be checking you closely to see if any side effects are occurring. Routine blood and urine tests will be done to monitor the effects of the investigational vaccine. Many side effects disappear after the drug is stopped. In the meantime, your health care provider may prescribe medications to keep these side effects under control.

There is no need for the use of contraceptive devices following injection of the adenovirus/PSA vaccine since the virus will not be present in the semen and cannot be transmitted to your partner.

The following summarizes the known risks of the Ad/PSA vaccine. There is still very limited experience with the vaccine and there could be other side effects we do not yet know about. As with any investigational product, there is always some chance of unexpected serious or even life-threatening side effects.

Likely/Common Risks (more than 35%)
Life Threatening – none
Serious – none
Mild – local tenderness, reddening, swelling at vaccination site

Less Likely/Less Common (10% - 35%)
Life Threatening – none

Serious – none
Mild – none

Rare (less than 10%)
Life Threatening – none
Serious – decrease in white blood cell count
Mild – flu or cold-like symptoms
protein leakage into urine
headache
fever

BLOOD DRAW

During the screening for eligibility and all follow up visits you will have blood drawn for testing. This may cause bruising or infection at the needle site and/or fainting. We will attempt to minimize these possible side effects by using a trained professional for all blood draws.

Likely/Common Risks (more than 35%)
Life Threatening – none
Serious – none
Mild – local tenderness, reddening, swelling at blood draw site

Less Likely/Less Common (10% - 35%)
Life Threatening – none
Serious – none
Mild – none

Rare (less than 10%)
Life Threatening – none
Serious – none
Mild – none

ARE THERE ANY UNFORESEEN RISKS?

In addition to the risks described above, there may be unknown risks, or risks that we did not anticipate, associated with being in this study.

WHAT ARE THE BENEFITS OF THIS STUDY?

We don't know if you will benefit from being in this study. However, we hope that, in the future, other people might benefit from this study as data obtained from this study may indicate the level of benefit obtained from the vaccination.

WHAT OTHER TREATMENT OPTIONS ARE THERE?

Before you decide whether or not to be in this study, your study physician will discuss other options that are available to you. Instead of being in this study, alternatives which could be considered in your case may include hormonal therapy if you are not already on this treatment. Taxanes have also shown to have limited benefit for some patients. Your study physician can provide detailed information about your disease and the benefits of alternative therapies. You should feel free to discuss you case and prognosis with your health care provider.

WILL IT COST ME ANYTHING TO BE IN THIS STUDY?

There may be additional costs for being on this study. These include time lost at work and travel expenses. The vaccine Ad/PSA,the costs of administration, and your stay in the GCRC will be provided to you without cost. You and/or your insurance company will not be billed for any procedures related specifically to this study.

You and/or your medical/hospital insurance carrier will remain responsible for your regular medical care expenses.

WILL I BE PAID FOR PARTICIPATING?

Other than medical care that may be provided and any other payment specifically stated in the consent form, there is no other compensation available for your participation in this research.

WHO IS FUNDING THIS STUDY?

The U.S. Department of Defense (DOD) is funding this research study. This means that the University of Iowa is receiving payments the DOD to support the activities that are required to conduct the study. No one on the research team will receive a direct payment or increase in salary from the DOD for conducting this study.

WHAT IF I AM INJURED AS A RESULT OF THIS STUDY?

- If you are injured or become ill from taking part in this study, medical treatment is available at the University of Iowa Hospitals and Clinics.
- No compensation for treatment of research-related illness or injury is available from the University of Iowa unless it is proven to be the direct result of negligence by a University employee.
- If you experience a research-related illness or injury, you and/or your medical or hospital insurance carrier will be responsible for the cost of treatment.
- If you are hurt or get sick because of this research study, you can also receive medical care at an Army hospital or clinic free of charge. You will only be treated for injuries that are directly caused by the research study. The Army will not pay for your transportation to and from the hospital or clinic. If you have questions about this medical care, talk to the principal investigators for this study, (David M. Lubaroff, PhD, 319-335-8423 or Richard D. Williams, MD, 319-356-0760). If you pay out-of-pocket for medical care elsewhere for injuries caused by this research study, contact Drs Lubaroff or Williams. If the issue cannot be resolved, contact the U.S. Army Medical Research and Materiel Command (USAMRMC) Office of the Staff Judge Advocate (legal office) at (301) 619-7663/2221.
- In the event of an emergency you should seek immediate care by calling 911, your local physician, the University of Iowa (319-356-1616) or Iowa City VA Medical Center urology staff on call (319-338-0581).

WHAT ABOUT CONFIDENTIALITY?

We will keep your participation in this research study confidential to the extent permitted by law. However, it is possible that other people may become aware of your participation in this study. For example, federal government regulatory agencies, the National Institutes of Health/Office of Biologic Activities (NIH/OBA), the Department of Defense, the U.S. Food and Drug Administration, auditing departments from the University of Iowa, and the University of Iowa Institutional Review Board (a

committee that reviews and approves research studies) may inspect and copy records pertaining to this research. Some of these records could contain information that personally identifies you.

In the future, the granting agency may continue to use your health information that is collected as part of this study. For example, they may combine information from this study with the results of other studies to re-analyze the safety and effectiveness of the study medication, to evaluate other products or therapies, to develop a better understanding of a disease, or to improve the design of future research studies. The funding agency may also share information from this study with regulatory agencies in foreign countries.

To help protect your confidentiality, we will assign a specific coded number to your file that will appear on the data forms and files, all written files will be kept in a locked office and information on computers will be protected by secure passwords. Only your University of Iowa Hospitals and Clinics or VA Medical Center records will contain information that associates your name with the research study. A sample of your blood will be sent to the Mayo Clinic for special laboratory testing. These blood samples will only be identified by a coded trial specific code. Only the University of Iowa and VA Medical Center investigators will know your identity and will not disclose that information to our Mayo Clinic colleagues. If we write a report or article about this study or share the study data set with others, we will do so in such a way that you cannot be directly identified.

A copy of the Informed Consent Document will be placed in your medical record.

WILL MY HEALTH INFORMATION BE USED DURING THIS STUDY?

The Federal Health Insurance Portability and Accountability Act (HIPAA) requires the University of Iowa Health Care or Iowa City VA Medical Center to obtain your permission for the research team to access or create “protected health information” about you for purposes of this research study. Protected health information is information that personally identifies you and relates to your past, present, or future physical or mental health condition or care. We will access or create health information about you, as described in this document, for purposes of this research study and for your treatment. Once the University of Iowa Health Care or Iowa City VA Medical Center has disclosed your protected health information to us, it may no longer be protected by the Federal HIPAA privacy regulations, but we will continue to protect your confidentiality as described under “Confidentiality.”

We may share your health information related to this study with other parties including federal government regulatory agencies, the University of Iowa Institutional Review Boards and support staff,

You cannot participate in this study unless you permit us to use your protected health information. If you choose *not* to allow us to use your protected health information, we will discuss any non-research alternatives available to you. Your decision will not affect your right to medical care that is not research-related. Your signature on this Consent Document authorizes the University of Iowa Health Care to give us permission to use or create health information about you.

Although you may not be allowed to see study information until after this study is over, you may be given access to your health care records by contacting your health care provider. Your permission for us to access or create protected health information about you for purposes of this study has no expiration date. You may withdraw your permission for us to use your health information for this research study by sending a written notice to David M. Lubaroff, PhD, Department of Urology, 200 Hawkins Drive, Iowa City, IA 52242. However, we may still use your health information that was collected before withdrawing your permission. Also, if we have sent your health information to a third

party, such as the study sponsor, or we have removed your identifying information, it may not be possible to prevent its future use. You will receive a copy of this signed document.

IS BEING IN THIS STUDY VOLUNTARY?

Taking part in this research study is completely voluntary. You may choose not to take part at all. If you decide to be in this study, you may stop participating at any time. If you decide not to be in this study, or if you stop participating at any time, you won't be penalized or lose any benefits for which you otherwise qualify.

WHAT IF I DECIDE TO DROP OUT OF THE STUDY?

If you decide to leave the study early, we will ask that you meet with one of the research study team members to provide the reasons for withdrawal. We will, of course, honor your request and there will not be any consequences to your withdrawal.

WILL I RECEIVE NEW INFORMATION ABOUT THE STUDY WHILE PARTICIPATING?

If we obtain any new information during this study that might affect your willingness to continue participating in the study or directly affect your continued health, we will promptly provide you with that information.

CAN SOMEONE ELSE END MY PARTICIPATION IN THIS STUDY?

Under certain circumstances, the researchers or the study sponsor might decide to end your participation in this research study earlier than planned. This might happen for any one or more of the following reasons: (a) in our judgment it would not be safe for you to continue, (b) because your condition has become worse, (c) because funding for the research study has ended, (d) the data and safety monitoring committee has closed the trial due to a low number of subjects entered into the trial..

WHAT IF I HAVE QUESTIONS?

We encourage you to ask questions. If you have any questions about the research study itself, please contact: Richard D. Williams, MD at (319) 356-0760 or Dr. Fadi Joudi at (319) 384-5993 of the Department of Urology, or Dr. Daniel Vaena (319) 356-1616 of the Department of Internal Medicine Hematology/Oncology. If you are calling after hours please call 319-356-1616 and ask for the Urology Resident or Oncology Fellow on call.

If you have questions about the rights of research subjects or research related injury, please contact the Human Subjects Office, 300 College of Medicine Administration Building, The University of Iowa, Iowa City, Iowa, 52242, (319) 335-6564, or e-mail irb@uiowa.edu. General information about being a research subject can be found by clicking "Info for Public" on the Human Subjects Office web site, http://research.uiowa.edu/hso.

FOR IRB USE ONLY
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$STAMP_EXP_DT

This Informed Consent Document is not a contract. It is a written explanation of what will happen during the study if you decide to participate. You are not waiving any legal rights by signing this Informed Consent Document. Your signature indicates that this research study has been explained to you, that your questions have been answered, and that you agree to take part in this study. You will receive a copy of this form.

Subject's Name (printed):

_____

Do not sign this form if today's date is on or after $STAMP_EXP_DT.

_____ _____

(Signature of Subject) (Date)

Permanent Address (printed): _____

_____

Statement of Person Who Obtained Consent

I have discussed the above points with the subject or, where appropriate, with the subject's legally authorized representative. It is my opinion that the subject understands the risks, benefits, and procedures involved with participation in this research study.

_____ _____

(Signature of Person who Obtained Consent) (Date)

UNIVERSITY of IOWA HEALTH CARE

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August 16, 2007

Dr----------

Dear Dr.---------,

Over the past 10 years, we at the University of Iowa along with the help of many of our colleagues all over the state have been investigating **gene transfer as an investigational approach for prostate cancer using an adenovirus vaccine carrying the PSA gene**. This approach is based on the concept that exposing prostate cancer patients to the exogenously derived PSA vaccine can activate/augment the cell mediated immune response against PSA which would then act against all PSA producing cells in the body. The Ad5PSA vaccine has proven very effective in causing the death of prostate cancer cells in tissue culture as well as in limiting or even abrogating the growth of prostate tumors injected into mice.

We have completed a Phase I clinical trial of the vaccine and are now initiating a P**hase II clinical trial at the University of Iowa and the Iowa City VA Medical Center.** This Phase II trial is aimed at establishing the ability of the Ad5PSA therapy to induce strong anti-PSA and hence, anti-prostate cancer immune response which we propose will induce a therapeutic benefit to the patients. In the Phase I study we treated a total of 32 patients. The main side effects we encountered included a transient erythema and tenderness at the injection site lasting a few days to 2 weeks and some flu or cold like symptoms. A few patients experienced transient proteinurea, and one patient had a reduced ANC. Whereas the Phase I trial used only a single injection of the vaccine this Phase II trial will use three injections. Therefore, the side effects seen previously may not be indicative of the higher dose to be used in this trial. We will enroll patients into one of two protocols. In the first protocol we will treat men with recent evidence of recurrent disease and in the second protocol we will treat men with hormone refractory disease.

We request your help in identifying additional patients for this study. If you have any patients who you feel may be interested in participating in the trial please ask them to contact Tammy Madsen, PA, our study co-coordinator at 319-356-3850 or Carlene Etscheidt, MSN at 319-356-1228. Feel free to contact Dr. Fadi Joudi (319-384-5993), Dr. Richard Williams (319-356-0760), Dr. Mark Smith (319-384-61350, or Dr. Daniel Vaena (319-338-0581, ext. 5212) if you have questions. After the patient contacts our team we will mail a copy of the Informed Consent document for him to read and to become familiar with the trial. We will then follow up with a call to the patient to arrange for his visit to the UIHC or ICVAMC where he will sign the consent document and undergo screening for eligibility. Should your patient decide to be screened for the trial, you will need to have him sign a HIPAA release form permitting our team to access his disease-specific medical record. We will only ask for permission to examine the medical record after the patient has provided verbal consent during a telephone call with a member of our research team.

We look forward to your cooperation in identifying patients so we may complete our Phase II trial in a reasonable time frame and determine the efficacy of this gene therapy for the treatment of recurrent prostate cancer.

Best regards,

Richard D. Williams, MD
Study Co-Principal Investigator

Fadi Joudi, MD
Study Co-Investigator

Daniel Vaena, MD
Study Co-Investigator

David Lubaroff, PhD
Study Principal Investigator

Mark C. Smith, MD
Study Co-Investigator

Eligibility Criteria

Protocol #1 -

Inclusion Criteria:

Men with prostate cancer who have received prior local therapy (radical prostatectomy or definitive radiation therapy) and have biochemical (PSA) relapse without evidence of radiographic or clinical metastatic disease.

For men who had prior prostatectomy, the surgery must have occurred at least 6 months prior to study enrollment.

For men who had prior definitive radiation therapy, radiation must have occurred at least 1 year prior to study enrollment.

Exhibit at least four separate rises in serum PSA, at least one month apart with differences $\geq$0.03 ng/ml and a total PSA of >0.2 ng/ml.

Have a PSA doubling time of $\geq$6 months.

Not at high risk as defined as those with a serum PSA of >20 ng/ml and a Gleason score of >7 before prostatectomy or radiation.

Negative bone scans.

Negative CT scans of chest abdomen and pelvis (no soft tissue metastases present).

Scans must be obtained within 6 weeks of entry into the trial.

Written informed consent.

Age $\geq$ 18 years.

Required laboratory values (obtained within 2 weeks of study entry)

Serum creatinine $\leq$ 2.0 mg/dL

Adequate hematologic function: granulocytes $\geq$ 1800 per mm^3, platelets $\geq$ 100,000 per mm^3, WBC $\geq$3700, and lymphocytes $\geq$590.

Adequate hepatocellular function: AST <3x normal and bilirubin <1.5 mg/dl.

Exclusion criteria:

Candidates for salvage radiation therapy unless the patient refuses.

Had a serum PSA of >20 ng/ml prior to surgery or radiation.

Gleason score of >7.

Seminal vesicle involvement or positive lymph nodes.

Active or unresolved infection.

Parenteral antibiotics <7 days prior to study entry.

Evidence of prior or current CNS metastases. Specific imaging is not necessary in the absence of signs or symptoms.

Co-morbid medical conditions which would result in a life expectancy (participation) of less than 1 year.

Patients with compromised immune systems; congenital, acquired, or drug-induced (immunosuppressive agents) will be excluded from the study. Use of prednisone at doses higher than 10 mg daily (or equipotent steroid doses) for more than 7 days within the last 3 months is not allowed.

No-pre-existing malignancies that required treatment within the past 5 years except for basal or squamous cell cancers of the skin.

Prior systemic therapies for prostate cancer not allowed (hormonal therapy, including but not limited to LHRH agonists, antiandrogens, ketoconazole or chemotherapy – mitoxantrone/taxanes/estramustine, etc.); only patients in Arm B, undergoing androgen depletion therapy during the vaccination will be eligible.

Prior participation in any vaccine studies for any disease.

The inability to understand the language and the clinical protocol.

Allergy or religious objection to pork products; Gelfoam is produced from pork.

Protocol #2

Inclusion criteria:

Men with prostate cancer who present with evidence of hormone refractory disease (D3).

Men with a positive bone scan, a PSA doubling time of $\geq$12 months, and a total PSA of <5 ng/ml, and asymptomatic.

Men with a negative bone scan with any PSA doubling time and asymptomatic.

Scans must be obtained within 6 weeks of entry into the trial.

Written informed consent.

Age $\geq$ 18 years.

Required laboratory values (obtained within 2 weeks of study entry)

Serum creatinine $\leq$ 2.0 mg/dL

Adequate hematologic functions: granulocytes $\geq$ 1800 per mm^3, platelets $\geq$ 100,000 per mm^3, WBC $\geq$3700, and lymphocytes >590.

Adequate hepatocellular function: AST <3x normal and bilirubin <1.5 mg/dl.

Castrate levels of testosterone of <5 ng/ml.

Exclusion criteria:

Active or unresolved infection.

Parenteral antibiotics <7 days prior to study entry.

Evidence of prior or current CNS metastases. Specific imaging is not necessary in the absence of signs or symptoms.

Co-morbid medical conditions which would result in a life expectancy (participation) of less than 1 year.

Patients with compromised immune systems; congenital, acquired, or drug-induced (immunosuppressive agents) will be excluded from the study. Use of prednisone at doses higher than 10 mg daily (or equipotent steroid doses) for more than 7 days within the last 3 months is not allowed.

No-pre-existing malignancies that required treatment within the past 5 years except for basal or squamous cell cancers of the skin.

Prior participation in any vaccine studies for any disease.

Prior chemotherapy, defined as prior cytotoxic chemotherapy for prostate cancer (or any cancer unless more than 5 years have elapsed). Examples of cytotoxic chemotherapy are mitoxantrone/prednisone and taxanes. Drugs such as Casodex or ketoconazole treatment must have been completed at least 6 weeks prior to registration.

The inability to understand the language and the clinical protocol.

Allergy or religious objection to pork products; Gelfoam is produced from pork.